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**Pharmaceutical self-micro-emulsifying lipid formulations to improve the bioavailability of poorly water-soluble drugs**

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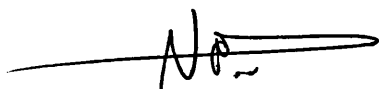
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# Pharmaceutical Self-Micro-emulsifying Lipid Formulations To Improve the Bioavailability Of Poorly Water-Soluble Drugs

Submitted by Naser M. Y. Hasan (BSc, MPhil)  
for the degree of  
Doctor of Philosophy (PhD)  
of the University of Bath  
October 2004

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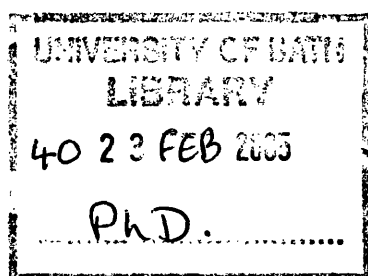
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*Soon We will show them our signs  
in the horizons and in their own souls,  
until it becomes manifest to them that this the truth.*  
Is it not enough that your Lord does witness all things?

**Qur'an**  
(Chapter 41, Verse 53)

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## Abstract

In recent years there has been growing interest in the self-emulsified lipid technology as an approach for the oral delivery of hydrophobic compounds. The use of combinatorial chemistry in synthesizing active molecules has produced wide range of drugs with high molecular weight and poor water solubility. The route for tablet formulation restricts the bioavailability of these compounds due to their low intrinsic water solubility and hence slow dissolution rate which is a key step in the absorption of these compounds. Therefore, if a rapid onset action is required the choice for tablet formulation is not an efficient approach. Various formulation methods have been attempted to improve the bioavailability of lipophilic compounds of which, self-emulsifying drug delivery systems (SEDDS) exhibit the most successful approach. Dissolution and disintegration steps are not necessary for drugs formulated in SEDDS as they are presented to the GIT in a soluble form dissolved within the lipid matrix, and ready for absorption after the emulsification process takes place. In order to facilitate dealing with these lipid systems, they were classified into type I, II, IIIA and IIIB based on various physicochemical factors. In the design for a successful lipid formulation, various key elements in the composition have to be optimized to obtain dispersions with minimum emulsion droplet size, less sensitive to the presence of electrolytes in the emulsification media and are able to maintain the drug in solution after the dispersion of the formulation. These elements include; type of oil, the use of cosurfactant (mixed mono/di-glycerides), oil-cosurfactant ratio, type of surfactant and the inclusion of cosolvents. In this study a self-emulsifying type II formulation was developed composed of Miglyol 812 (medium chain triglycerides), Imwitor 988 (medium chain mono/di-glycerides) and the non-ionic surfactant Tagat TO. The constituents of this system are considered to be water insoluble materials which make the formulation highly unlikely to lose its solvent capacity after dispersion. Very fine dispersions of droplet size between 50 and 60nm which are arbitrarily called o/w microemulsions were obtained at Miglyol 812/Imwitor ratios of 70/30 or 60/40 and at Tagat TO concentration of 30% w/w. Phase behaviour studies of these systems have shown regions of no liquid crystalline material (LC) yet, extended areas of water solubilization ( $L_2$ ) was observed. This indicates that 'Diffusion and Stranding' theory is the putative mechanism for the emulsification process in these systems. These systems appear to be prone to the effect of electrolytes present in the emulsification media. Furthermore, Physical characterization of resultant dispersions of type III self-micro-emulsified lipid system composed of Miglyol 812/Imwitor 988-Cremophor RH40 was carried out. Optimum dispersions of  $\approx 30$ nm were obtained using oil blends of Miglyol 812/Imwitor 988 at ratios of 1:1 and Cremophor RH40 at concentration of 30% w/w. In order to find alternatives to Cremophor RH40 because of its waxy nature at ambient temperature, various hydrophilic surfactants were screened for microemulsion systems. Self-micro-emulsifying systems were obtained using blends of Miglyol 812/Imwitor 988 at ratios of 1:1 in the case Cremophor EL, and at ratio of 2:3 in the case of Crillet 4 or Tagat O2. The inclusion of water-soluble cosolvents in SEDDS formulations was found to accelerate precipitation of drug after dispersion depending on the emulsification media. Oil-cosurfactant ratio, type of surfactant and the amount of included water-miscible cosolvents can influence the solubilization behaviour of the drug after dispersion. Finally, Solid self-micro-emulsified lipid systems (SMELS) were developed from eutectic mixtures of various solid carrier systems and oil mixtures. These new vehicles have the potential of presenting the drug to the GIT in the amorphous state with no susceptibility to the aging processes.

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## Origin and Scope of this Study

The use of Self-emulsifying drug delivery systems (SED DS), as an approach to improve the bioavailability of poorly water-soluble drugs, was first initiated by the work of Pouton in 1982 <sup>[8]</sup> on various mixtures of oils and surfactants. Self-emulsifying formulations are considered to be mixtures of oils and surfactants which upon gentle agitation in water to simulate physiological conditions in the stomach spontaneously emulsify forming dispersions of droplet size of  $<5\mu\text{m}$ . Pouton in his work identified a self-emulsifying system composed of Miglyol 812 (medium chain triglyceride) and Tween 85 (polyoxythelene-(25)-sorbitan trioleate). Later, Wakerly <sup>[9]</sup> working under the supervision of Pouton identified a system which satisfied the parameters of self-emulsifying lipid systems composed of Miglyol 812 and Tagat TO (polyoxythelene-(25)-glycerol trioleate). Thorough studies on those two systems have revealed the formation of liquid crystalline material which is involved in the mechanistics of emulsification in these formulations. Since then, SED DS have attracted the attention of many researchers and recently, culminated in the development of Sandimmune™ (cyclosporine A) as Neoral™ which is a vivid example of the advances in this technology. Neoral™ is considered a stereotypical type III self-micro-emulsifying drug delivery system (SMEDS) according to the classification suggested by Pouton <sup>[111]</sup>. Self-emulsifying systems have the potential advantage of presenting the active compound already dissolved in the lipid matrix ready for absorption. These systems skip the disintegration and dissolution processes which are rate-limiting steps in the absorption of traditional tablet formulations.

One of the major breakthroughs in this technology is the use of mixed mono and diglycerides (such as, Imwitors and Capmuls) in the oil blend as co-surfactants to aid in the emulsification process, and also the use of surfactants of high solubilizing capacity like Cremophors (polyoxyethylene castor oil derivatives).

The core theme of this project is divided into two parts which are presented respectively in chapter 3 and 4: Firstly; identifying and physical characterization of systems which are potential self-micro-emulsifying drug delivery systems (SMEDS) composed of oil blends of Miglyol 812/Imwitor 988 and various surfactant systems, investigating mechanistic processes involved in emulsification and exploring the fate

of dissolved drug after the dispersion of the formulation, and secondly; preparing self-micro-emulsified lipid systems as a solid dosage form in order to replace costly and inconvenient soft gelatin capsule forms.

## Chapter 1

### Introduction

#### 1.1 The Concept of Self-emulsifying Drug Delivery Systems (SEDDS): Formulation Perspective.

It is important to distinguish between self-emulsifying systems and conventional emulsion systems which will be finely dispersed only after exposure to strong shearing forces. A self-emulsifying system is a mixture of oil and surfactant which emulsifies in water under conditions of gentle agitation. Such mixtures may be spontaneously emulsified if the entropy change favoring dispersion is larger than the energy required to increase the surface area of dispersion <sup>[1]</sup>. Self-emulsifying formulations have been used by the herbicide and pesticide industries for several years <sup>[2, 3]</sup>. Recently, self-emulsifying systems have been formulated using medium-chain triglyceride oils and nonionic surfactants, which being acceptable for oral ingestion, could form the basis of self-emulsifying drug delivery systems <sup>[4-10]</sup>. These mixtures can be formulated into soft gelatin capsules to produce precise and convenient unit dose forms which, after oral administration, will emulsify within the gastric contents <sup>[11]</sup>. Lipid-based formulation can reduce the inherent limitation of slow and incomplete dissolution of poorly- water soluble drugs, and facilitate the formation of solubilised phases from which absorption may occur <sup>[12]</sup>. In theory the use of SEDDS for oral administration of hydrophobic drugs would appear advantageous however few exist. A significant limitation to their use is the effort involved in optimization of a SEDDS. An ideal SEDDS should disperse into an emulsion consisting of droplets <5 $\mu$ m in diameter with a uniform size distribution, to reduce the diffusion path of the drug from the droplet <sup>[4, 13, 14]</sup>. Such a formulation will present a lipophilic drug in oily solution with a large interfacial area across which diffusion can take place. The mechanisms, rate and extent of drug absorption from the resulting emulsion will be strongly dependent on the oils and surfactants used in each formulation. For example, absorption will be influenced by whether or not the oil is digestible and by the partitioning of drug between the oil and water <sup>[15]</sup>. Therefore, the

absorption profiles of a drug from a series of self-emulsifying formulations are likely to be different but it is hoped that the absorption from an individual formulation will be comparatively reproducible, leading to improved control of the bioavailability of lipophilic drugs from GIT. One advantage that self-emulsifying formulations have over solid dosage formulations is the avoidance of slow drug dissolution. In addition, distribution of the emulsion within the GIT may help to avoid the irritancy which can be caused by contact between bulk solids and the gut wall. Obviously, the excipients used for formulation would need to be non-irritant and free from other acute or chronic toxicity problems. Choice of oils is likely to be restricted to silicones, liquid paraffin or vegetable oils and other derivatives. These could be used in combination with non-ionic surfactants which are generally less toxic than ionic surfactants <sup>[16]</sup>. The efficiency of SEDDS depends on three main factors; particle size of oil droplets on exposure to aqueous media, rate of emulsification <sup>[4]</sup> and the polarity of the resulting oil droplets <sup>[14]</sup> which promotes partitioning of the drug into the aqueous phase. The polarity of the oil droplets is governed by the hydrophilic-lipophilic balance (HLB), the chain length and degree of un-saturation of the fatty acid, molecular weight of the hydrophilic portion and the concentration of the emulsifier.

The pharmaceutical application of self-emulsification requires the identification of surfactant-oil mixtures which might be pharmaceutically acceptable, be capable of good self-emulsification and be feasible of encapsulation in a soft gelatin capsule. Pouton <sup>[4]</sup> has identified a vegetable oil - nonionic surfactant mixture composed of Miglyol 812 or Miglyol 840, a medium chain triglyceride oil of vegetable origin, and Tween 85 (polyoxythelene-(25)-sorbitan trioleate) which satisfied these parameters. However, the self-emulsifying behavior of this system was found to be extremely sensitive to dissolved solutes in both the SEDDS and the aqueous test media. Within a range of more efficiently emulsifying mixtures (20-60 % w/w Tween 85), the shortest emulsification times occurred between 30 and 45% w/w Tween 85. A minimum in particle size of approximately 300nm for self-emulsified systems was produced by compositions of oil and surfactant containing 25-35% w/w Tween 85.

Wakerly *et al.* <sup>[7]</sup> screened a range of non-ionic surfactants and oils for self-emulsifying behavior. It was determined that unsaturated ester- based surfactants were more efficient at forming self-emulsifying systems than the corresponding saturated surfactants with either a medium chain triglyceride lipid or Archis oil. They studied

binary mixtures of Tagat TO and Miglyol 812 in very great detail, efficient self-emulsification was produced from mixtures containing 35 to 52.5% Tagat TO. Minimum particle size of 100-200 nm was achieved using approximately 50% surfactant concentration. Charman *et al.* <sup>[13]</sup> formulated and evaluated a self-emulsifying formulation, based on the system developed by Wakerly *et al* <sup>[7]</sup>, for the delivery of an investigational lipophilic anti-viral compound (WIN 54954). Their observations demonstrated that substantial proportions of WIN 54954 could be successfully incorporated into a self-emulsifying mixture with substantial improvement in bioavailability.

Many factors should be considered and optimized when formulating with lipid systems which will be discussed in more details in sections to come. This includes:

1. Emulsification rate and resultant particle size
2. Surfactant type and concentration
3. Electrolyte
4. Temperature
5. Inclusion of drug

## **1.2. Emulsions and Microemulsions**

### **1.2.1. Definition of Emulsions**

Over the last few decades, the production of emulsions has gained the interest of many scientists due to the wide range of technological products and processes involved. Stable emulsion systems are used in a large area of industry, such as the food industry, pharmacy, cosmetics and coatings. An emulsion may be defined as an opaque, heterogeneous system of two immiscible liquid phases (oil and water) where one of the phases is dispersed in the other as drops of microscopic or colloidal size, normally in the range 250nm-25 $\mu$ m <sup>[17]</sup>. There are two kinds of simple emulsions depending on which phase comprises the drops; if oil is dispersed in-water (O/W), water is called the continuous phase and oil is the dispersed phase; if water is dispersed in oil (W/O), oil is the continuous phase. Emulsions made by agitation of the pure immiscible liquids are very unstable as mixing increases the interfacial area resulting in an increase in the interfacial free energy, thus producing a

thermodynamically unstable system. Such emulsions can be stabilized by the addition of surface-active material which protects the newly formed drops from re-coalescence. An emulsifier is a surfactant which facilitates emulsion formation by reducing the system's interfacial tension and aids in stabilization through a combination of surface activity and possible structure formation at the interface.

Surfactants are available in many forms:

- Ionic surfactants: Cationic & Anionic

Quaternary ammonium alkyl salts form one of the best classes of cationic surfactants; amongst the most well known examples are:

- \* Cetyltrimethylammonium Bromide (CTAB) and
- \* Didodecyltrimethylammonium Bromide (DDAB)

One of the most commonly used anionic surfactant which is twin-tailed, and is particularly effective stabilizer in w/o microemulsions is:

- \* Sodium bis(2-ethylhexyl)-sulfocuccinate (AOT)

- Zwitterionic surfactants

A particular important class of this type is the naturally occurring Phosphoglycerides, which include Phosphatidylethanolamine (PE) and Phosphatidylcholine (PC).

- Nonionic surfactants

Examples of nonionic surfactants include

- \* Polyoxyethylenated fatty alcohol (Brij series)
- \* Sorbitan esters (Span series)
- \* Polyoxyethylene glycerol fatty acid esters (Tagat series)
- \* Hydrogenated castor oil ethoxylates (Cremophor series)
- \* Polyoxyethylene sorbitan esters (Tween series)

The safest surfactants to be used orally are the nonionic types and the most widely used are probably the ethoxylated esters of fatty acids such as Tweens and Spans. Nonionic surfactant molecules contain a polar hydrophilic group (polyoxyethylene chain) and a non-polar (lipophilic) chain. This amphiphilicity results in a double affinity which can only be satisfied at a polar-non-polar interface. Nonionic surfactant-oil-water systems (nSOW) are usually 'pseudo ternary' *i.e.* the surfactant may be a mixture of surfactants, the aqueous phase may be a salt solution and the oil phase may be a mixture of oils or contain dissolved polymers.

### 1.2.2. Definition of Microemulsions

It is possible to mix polar liquids such as water, glycerol or dimethylformamide <sup>[18]</sup> with non-polar hydrocarbons such as *n*-alkanes to form single-phase thermodynamically stable dispersions by the addition of a suitable surfactant or amphiphile. This phenomenon was reported to have been explained as early as 1943 by Hoar and Schulman <sup>[19]</sup> who proposed an “oleopathic hydro-micelle” model. Nonetheless, the term ‘microemulsion’ has been defined by Danielsson and Lindmann <sup>[20]</sup> as ‘a system usually consisting of oils, surfactants, cosolvent, and water which is a single phase, optically isotropic and thermodynamically-stable liquid solution’. The difference between a microemulsion and an ordinary emulsion is shown by the photograph in figure 1.1. Ordinary emulsions are milky turbid dispersions with particle size reaching few microns (see, vial C) whereas, microemulsions are optically clear dispersions of particle size ranging from 5-50nm (as in vials A & B). There are three types of microemulsions (a) oil-in-water microemulsion, (b) water-in-oil and (c) bicontinuous microemulsion. The difference between these types will be discussed in detail next. These systems have the capacity to incorporate typically 20% and up to approximately 40% volume fraction of dispersed phase in the continuous phase <sup>[22]</sup>.

### 1.2.3. Formation of Microemulsions

The main driving force for microemulsion formation arises from a reduction of the interfacial tension (interfacial free energy per unit area) which results from adsorption of surfactant at the oil water interface. The Gibbs free energy equation is shown in a simplified form in Equation 1, where  $\Delta G$  is the change in the Gibbs free energy (J) on dispersion,  $\Delta S$  is the entropy change ( $\text{JK}^{-1}$ ),  $T$  is the temperature in Kelvin,  $\Delta A$  is the increase in surface area of the interface ( $\text{m}^2$ ) and  $\gamma$  is the interfacial tension ( $\text{Nm}^{-1}$ ).

$$\Delta G = \gamma \Delta A - T \Delta S. \quad (1)$$

On mixing oil, water and surfactant to form a microemulsion there is an increase in  $A$  of several orders of magnitude compared to that in a phase separated system. In order to compensate for this effect there must be a dramatic reduction in the interfacial tension when surfactant is adsorbed at the interface. This is achieved essentially by coating the interface with surfactant molecules and forming a curved liquid-like

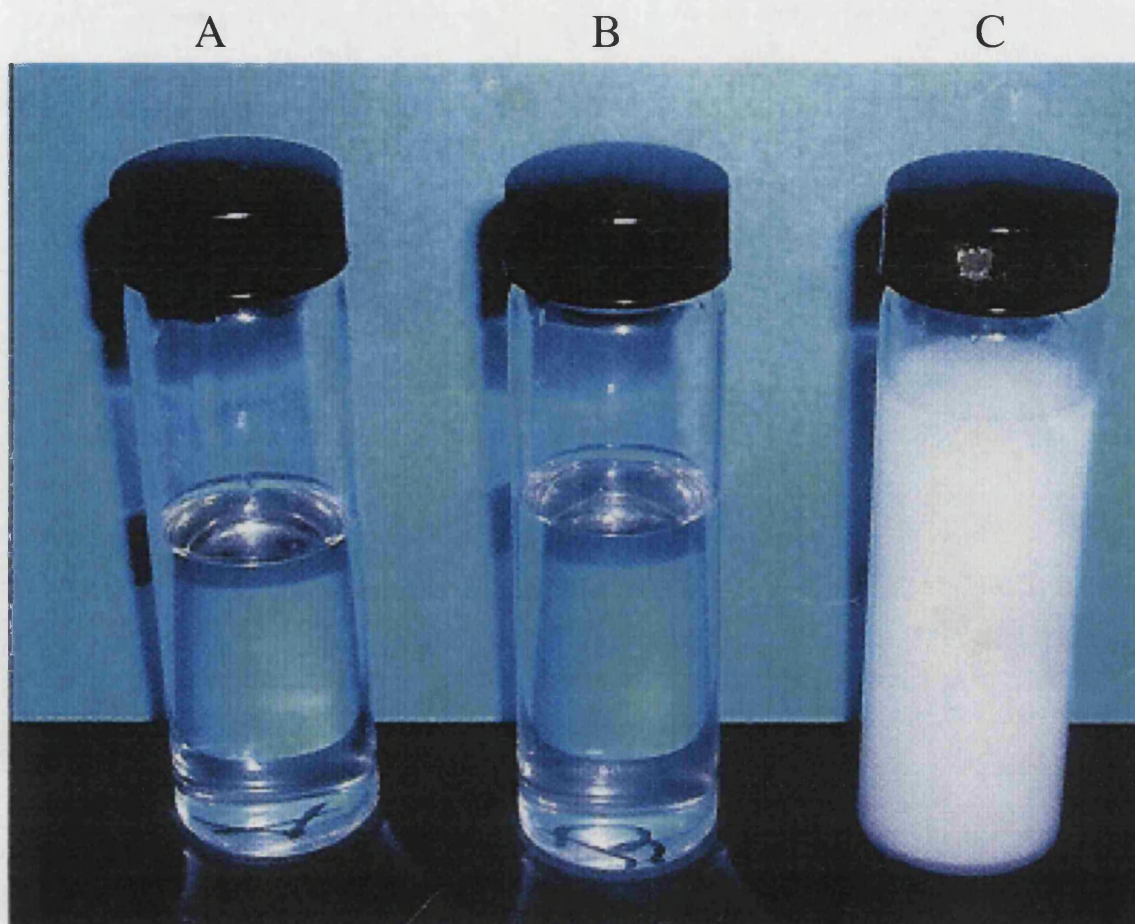


Figure 1.1

Photograph showing the difference between a microemulsion and an ordinary emulsion. (A) An emulsifier dissolved in oil with no added water (B) A microemulsion containing 15% water (C) An emulsion with 35% water (Adapted from Ref. 21).



monolayer film, thus preventing oil and water from coming into direct contact <sup>[23]</sup>. In this way, the interfacial tension is reduced from  $\approx 50 \text{ mNm}^{-1}$  to  $<1 \text{ mNm}^{-1}$ . However, entropy changes also influence the free-energy term. The dispersion of one liquid in the other in the form of large numbers of very small droplets will clearly increase the entropy of the system which is favorable. However, the adsorption of surfactant molecules at the oil-water interface might be expected to reduce the overall entropy increase. The latter unfavorable entropy change is thought to be reduced by the fluid-like nature of the interface. For example, lateral surfactant motion in the interface occurs, which decreases ordering in the surfactant monolayer. The net change in entropy associated with microemulsion formation is positive and therefore contributes to the lowering of the free energy term. Thus a negative free energy of formation is achieved when large reductions in surface tension are accompanied by significant favorable entropic change. In such cases, microemulsification is spontaneous and the resulting dispersion is thermodynamically stable.

The extent and position of the single-phase microemulsion region for a typical surfactant is shown in Figure 1.2a. In the oil rich domain of the single region (the right side shaded portion,  $L_2$  region) W/O microemulsion is preferred; towards the water rich corner ( $L_1$  region) O/W microemulsion is found. By their very nature O/W microemulsion droplets generally have a large effective interaction volume than W/O droplets. In the case of ionic surfactants this is attributable to the presence of the electrical double layer formed at the surface of the O/W droplet which introduces a strong repulsive force. Yet, in the case of nonionic surfactants, although there is hydration shell associated with polar headgroups, the predominant repulsive factor can be attributed to steric interactions. For single-phase mixtures of oil, water and surfactant which fall outside these well defined regions, it is possible that bicontinuous microemulsions may form, where the amounts of water, surfactant and oil are similar. In the latter case, both oil and water exist as a continuous phase in the presence of a continuously fluctuating surfactant-stabilised interface with a net curvature of zero. Figure 1.3 shows schematic representation of the three types of microemulsions discussed above which are most likely to be formed depending on composition. Bicontinuous systems are structurally more difficult to study but they may be regarded as a single-phase system in which both the oil and water have extensive connected domains so they behave as if both are continuous phase; *i.e.* there is no dispersed phase as such. Each phase may be regarded as occupying opposite

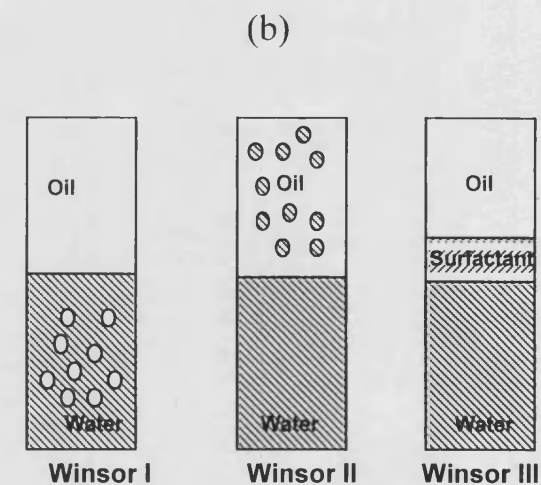
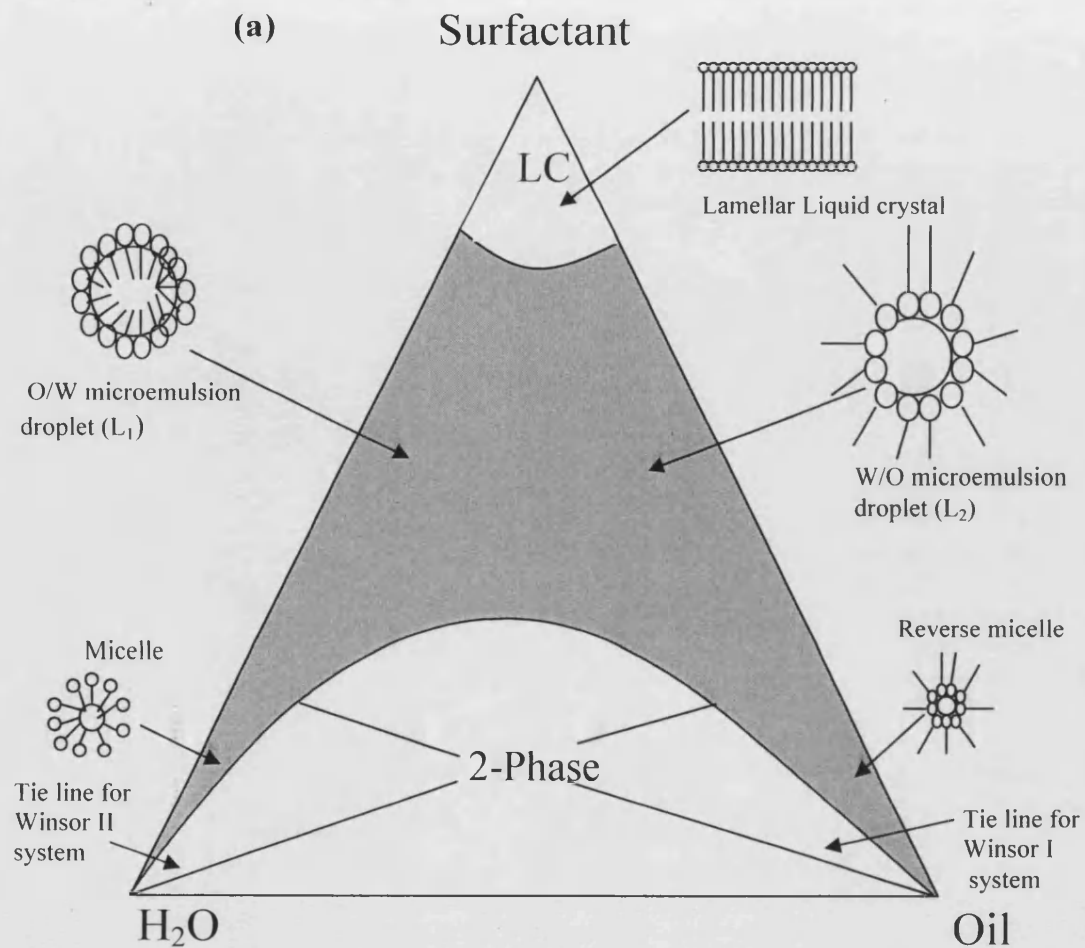


Figure 1.2b:

Winsor's description of the various types of the multiphase emulsion systems

Figure 1.2a: Schematic triangular phase diagram of an oil-surfactant-water system. Shaded area indicates existence of fields of the single phase microemulsion region: conventional micelles, reverse micelles, w/o and o/w microemulsions (adapted from Ref. 24).

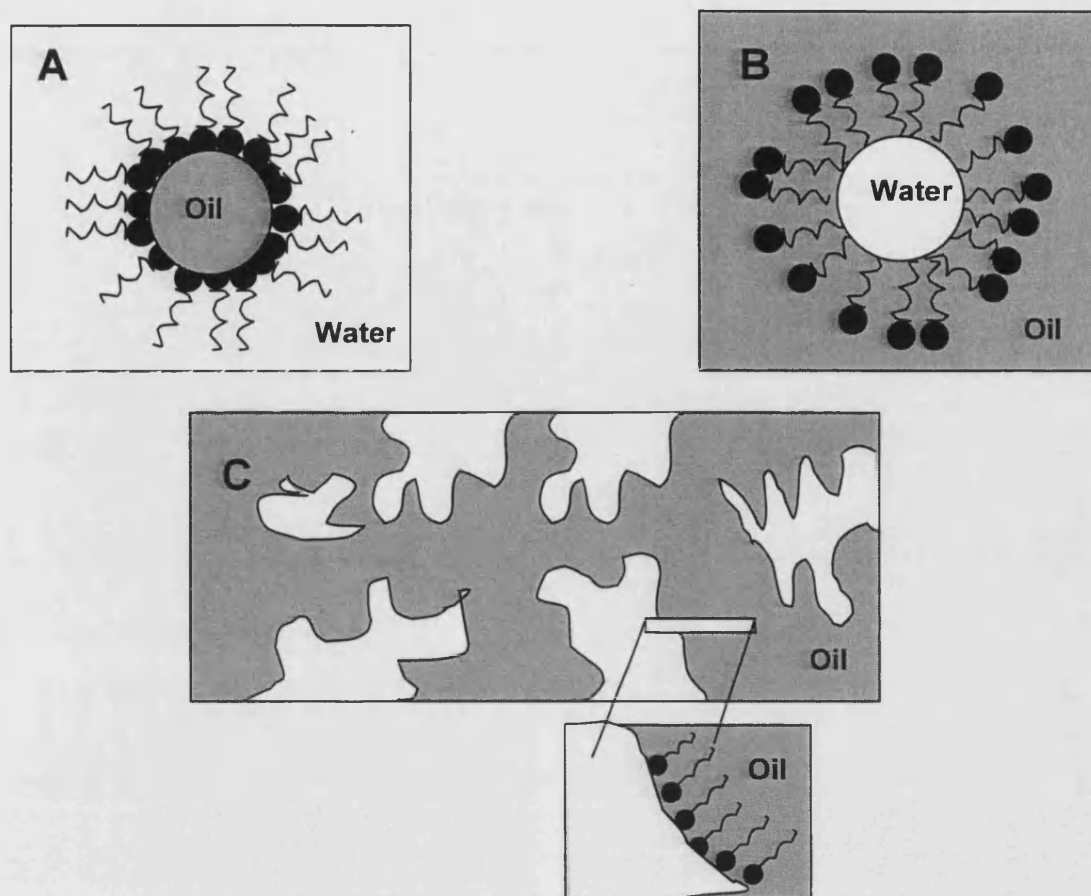


Figure 1.3

Schematic representations of the three most commonly encountered microemulsion types: (a) oil-in-water, (b) water-in-oil, and (c) Bicontinuous (adapted from Ref. 25).

sides of continuous, interconnecting, surfactant-coated channels <sup>[26]</sup>. Such bicontinuous microemulsions may possess relatively high viscosities compared to O/W or W/O microemulsions because of the presence of extended structures. For those microemulsions where droplets are present, the radii fall within the range 1-10nm. Early measurements were made using the sodium salt of Aerosol-OT as surfactant, the droplets being essentially monodisperse. Droplet sizes and shape are most commonly measured using scattering techniques such as small-angle neutron scattering (SANS) and photon correlation spectroscopy (PCS).

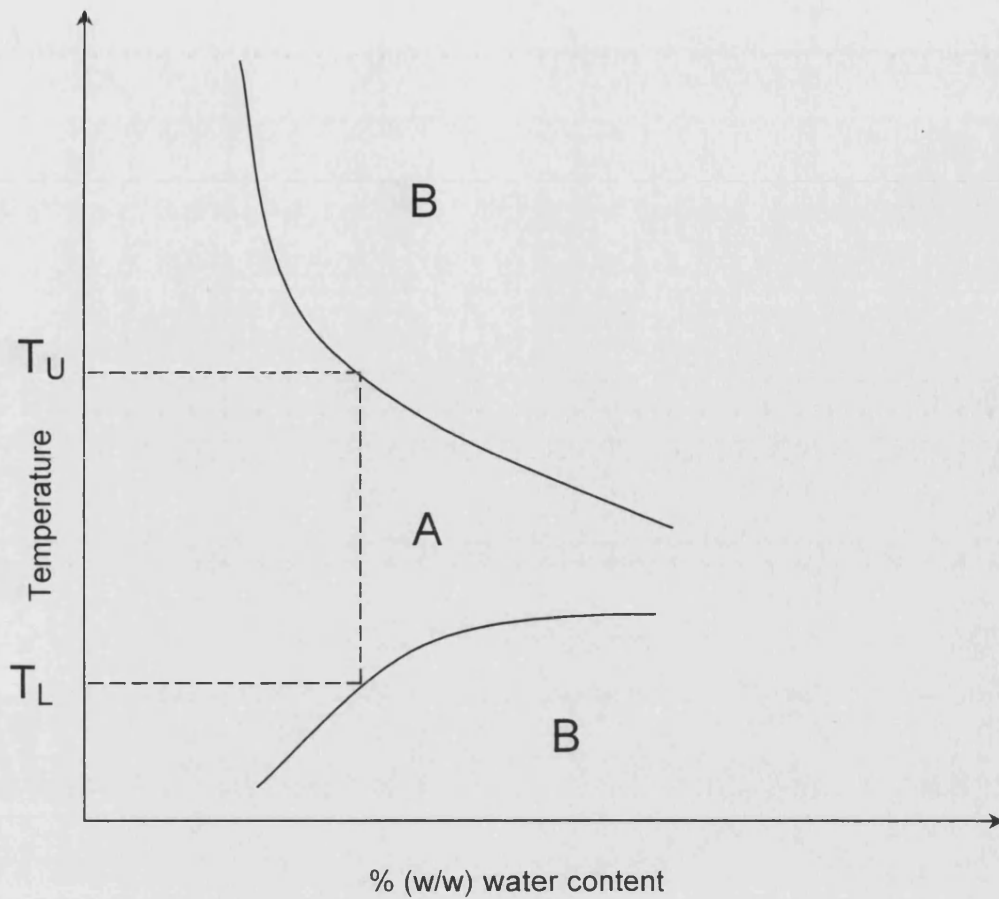
In certain systems microemulsion formation is achieved by adding with the surfactant, a suitable co-surfactant such as a medium chain-length alcohol (typically hexanol), alkanolic acids, alkanediols, alkyl amines <sup>[27]</sup>, medium chain-length ( $C_8/C_{10}$ ) mono/diglycerides such as Imwitor 988 as illustrated in this thesis, or even a co-solvent such as chloroform, propylene glycol etc. Co-surfactants act as surfactants in their own way; they stabilize the interface by keeping similarly-charged headgroups further apart, thus minimizing headgroup coulombic repulsions. The need for a co-surfactant is related to the fact that each head group unit is hydrophilic. Therefore, the addition of an extra unit causes a considerable increase in the hydrophilic nature of the surfactant. This makes it harder to achieve the correct HLB required for the production of a microemulsion; instead this balance has to be achieved by the use of a co-surfactant. Co-surfactants need not necessarily be capable of forming association structures on their own right. However, co-surfactants generally partition between the interface and the external oil phase. This means that their effect on the system, for example in changing the droplet size, depends on the partition coefficient of the co-surfactant concerned. Importantly, in some cases nonionic surfactants such as polyoxyethylene *n*-alkyl ethers ( $C_nE_m$ ) are able to form microemulsions without the need for co-surfactant <sup>[28, 29]</sup>. Although microemulsions appear at first to be rather complex fluids, their preparation is simple and straightforward. AOT microemulsions are readily prepared by dissolving the surfactant in the non-polar oil and then adding water with gentle shaking. This results in the formation of an optically-clear single-phase solution containing droplets.

Multiphase microemulsion-containing systems, first described by Winsor <sup>[30]</sup>, are also important. These are located in the two phase region of the phase diagram as shown in Figure 1.2a. The affinity of the surfactant for oil or water at a particular temperature

and or salinity results in three different types according to Winsor classification system, see figure 1.2b. Winsor's type I systems, the affinity of the surfactant for the water phase exceeds its affinity for the oil phase. Thus, the interface will be convex towards water. A system in the two-phase region will split into an oil phase containing dissolved surfactant monomers at  $CMC_o$  (critical micelle concentration in the oil phase) and an aqueous microemulsion—a water phase containing solubilised oil in normal surfactant micelles (O/W). In Winsor's type II systems, the affinity of the surfactant for the oil phase exceeds its affinity for the water phase and the interface will be convex towards oil. A system in the two-phase region will split into a water phase containing surfactant monomers at  $CMC_w$  (critical micelle concentration in the water phase) and an oleic microemulsion which is an oil phase containing solubilised water in reverse surfactant micelles (W/O). In Winsor's type III systems, the surfactant's affinity for the oil and the water phases is balanced. The interface will be flat as in bicontinuous microemulsions. In the multiphase region, type III nSOW (non-ionic surfactant-oil-water) system co-exists as a three phase-phase; a water phase containing surfactant monomers at  $CMC_w$ , an oil phase containing surfactant at  $CMC_o$  and a surfactant phase. The surfactant phase is composed of cosolubilised oil and water separated from each other by an interfacial layer of surfactant. This layer is sometimes called the middle phase as its intermediate density causes it to appear between the oil and the water phases in type III nSOW systems.

#### 1.2.4. Microemulsion Stability

A convenient and commonly encountered means of representing the thermal stability of a single-phase microemulsion system is the use of phase profile (Figure 1.4). The plot is generally one of  $R$  (% w/w water content) versus temperature for a microemulsion of fixed surfactant concentration. The phase profile defines the maximum amount water that can be solubilised in the oil solvent at any particular temperature. Also, the temperature range over which a given microemulsion is stable, limited by the upper and lower temperature phase boundaries,  $T_U$  and  $T_L$ , respectively. The schematic stability diagram shown in Figure 1.4 would be typical for sodium bis(2-ethylhexyl)-sulfocuccinate (AOT)/water/*n*-alkane mixtures and also for nonionic surfactants/water/mixed glycerides (optimized ratios of medium chain triglycerides/mono-diglycerides) as will be presented later in this thesis.



**Figure 1.4**

Schematic stability diagram of water content  $\%(\text{w/w})$  versus temperature for a single-phase emulsion. **A** represents the single-phase microemulsion. **B** represents the Regions where the system is phase-separated (adapted from Ref. 24).  $T_U$  and  $T_L$  are, respectively, the upper and lower temperature phase boundaries over which a given microemulsion is stable.

One distinguished feature of this phase profile is the fact that, a single-phase microemulsion system is not associated with the formation of liquid crystalline structures.

A major consideration in the stability of microemulsions is the miscibility behavior of the two-component surfactant-water and surfactant-oil systems. It is worth noting that ionic surfactants such as AOT and nonionic surfactants such as  $C_{12}E_6$  (hexaethyleneglycolmono-*n*-dodecyl ether) behave in opposite ways with temperature. In the case of AOT, the conversion is Winsor II to Winsor I with increasing temperature, whereas the change for  $C_{12}E_6$  is Winsor I to Winsor II. This could be attributed to the fact that microemulsions stabilized by non-ionic surfactants are very susceptible to temperature because a decrease in surfactant solubility occurs with increasing temperature. In contrast, microemulsions stabilized by ionic surfactants have little or no sensitivity to temperature <sup>[25]</sup>. The phase inversion of Winsor I to Winsor II or *vice versa* can be induced not only by a change in temperature but also by changes in ionic strength or through the addition of additives such as co-surfactants.

### 1.3. Basic Principles of Phase Inversion

The nature of an emulsion (O/W or W/O) depends on variable factors such as, oil:water ratio, electrolyte concentration, temperature and mainly the nature of the surfactant. If the surfactant is more soluble in the water phase than in the oil phase (hydrophilic surfactant) O/W emulsion is produced. On the other hand, if the surfactant is more soluble in the oil phase (lipophilic surfactant) W/O emulsion is formed. In order to explain the behavior of emulsified systems, three basic concepts related to the surfactants need to be understood: solubility, formation of micelles and interfacial adsorption.

#### 1.3.1. Dissolution State of Nonionic Surfactant

The most important properties of a surfactant are its solubility and its interfacial activity. When a nonionic surfactant is added to a two-phase liquid-liquid system, it preferentially adsorbs at the interface forming an adsorbed layer. At low surfactant

concentrations an equilibrium exists between surfactant monomers dissolved in the oil phase, surfactant monomers dissolved in the water phase and the interfacial surfactant. In the case of a separated system (constant interfacial area), as the concentration of surfactant in the system increases, the amount of surfactant at the interface reaches a maximum possible concentration. Excess surfactant, on further increase in surfactant concentration, will form micelles in either the oil or aqueous phase, or form a surfactant phase depending on its affinity for the oil and water phases. The break point is termed the critical micelle concentration (CMC). A surfactant micelle is generally depicted as sphere of surfactant molecules with a liquid phase core. The surfactant must be present at a concentration above the CMC in order to stabilize an emulsion.

### **1.3.2. Change of Phase Behavior with Temperature**

One way of altering the surfactant affinity in an nSOW system is by changing the temperature. This change in temperature will change the surfactant's affinity for the two phases. At high temperature the nonionic surfactant become more soluble in the oil phase while at low temperature it becomes more soluble in the water phase. Shinoda and Friberg <sup>[31]</sup> showed that, for surfactants with cyclohexane and water, the phase behavior changed with temperature at a constant surfactant concentration. In that system, a rise in temperature increased the surfactant's affinity for the oil phase, hence the system moved from Winsor's type 1 to type 2 phase behavior with increase temperature. The water phase was continuous at low temperature. Satio and Shinda <sup>[32]</sup> also showed that the interfacial tension changed during the transition from type 1 to type 2 phase behavior.

### **1.3.3. Factors Affecting Phase Inversion**

The phase inversion in nSOW systems has several controlling factors. Among these factors the following can be identified:

(1) Type of oil, (2) Surfactant type and concentration, (3) Temperature of the system, (4) Water to oil ratio, (5) Additives in the oil and water, (6) Mixing conditions and (7) Rate and order of additives of the different components; the first five factors affect the surfactant's affinity while the last two factors are dynamic variables.



Various techniques and concepts have been used to correlate surfactant affinity variables and hence the emulsion type; these are described below:

- A. Hydrophile-Lipophile Balance (HLB)
- B. Phase Inversion Temperature (PIT)
- C. Emulsion Inversion Point (EIP)
- D. Equilibrium Water-Oil Ratio (WOR) Maps

The first two will be discussed in this thesis.

### 1.3.3.1. Hydrophile-Lipophile Balance (HLB)

Full descriptions of the Hydrophile-Lipophile Balance (HLB) concept are given by Becher <sup>[17]</sup>, and Becher and Shick <sup>[33]</sup>. Griffin <sup>[34]</sup> first defined the affinity of a non-ionic surfactant in terms of an empirical quantity, the HLB. Surfactants are assigned an HLB number at 25°C on a scale of 1 to 20. Low HLB numbers represent lipophilic surfactants and high HLB numbers represent hydrophilic surfactants. Application of a surfactant can be derived from its HLB number in accordance with table 1.1 <sup>[35]</sup>.

HLB numbers are calculated for a surfactant from simple formulae based either on analytical or composition data <sup>[36]</sup>. For polyoxyethylene nonylphenyl ethers (NPE),  $HLB = E/5$  where  $E$  is the % weight of polyoxyethelene in the surfactant. For example, for NPE12 (12 oxyethylene groups in the hydrophilic chain)  $HLB = 14.2$ . For a polyhydric fatty acid ester,  $HLB = 20(1 - S/A)$ , where  $S$  = saponification number of the ester and  $A$  = acid number of the fatty acid. For example, for polyoxyethylene (20) sodium monolaurate (Tween 20),  $S = 45.5$  and  $A = 276$ ;  $HLB = 16.7$ .

**Table 1.1: Application of surfactant based on their HLB number**

<i>HLB number range</i>	<i>Application</i>
3-6	W/O emulsifier
7-9	Wetting agent
8-12	O/W emulsifier
13-15	Detergent
15-18	Solubiliser

Many attempts have been done to assign HLB numbers to various oils to predict which surfactants will produce the most stable emulsion *i.e.* the required HLB. However, many researchers have found no correlation between emulsion type and HLB number of surfactant

### 1.3.3.2. Phase Inversion Temperature (PIT)

The sensitivity of emulsions to temperature was recognized by Shinoda *et al* [37, 38]. They suggested the use of the PIT as a method of preparing emulsions. They found that at the PIT oil-water interfacial tension is at its minimum and the oil water interface does not show preferential curvature. Phase Inversion Temperature (PIT) was defined by Friberg *et al.* [39] as being the temperature at which the emulsifier shifts its preferential solubility from water to oil phase at high temperatures. Below and above the PIT the interfacial curvature is towards the oil and water respectively forming o/w or w/o emulsions. The PIT is a characteristic feature of an emulsion system which is influenced by specific non-ionic surfactant- oil and water components. Phase inversion temperature is affected by:

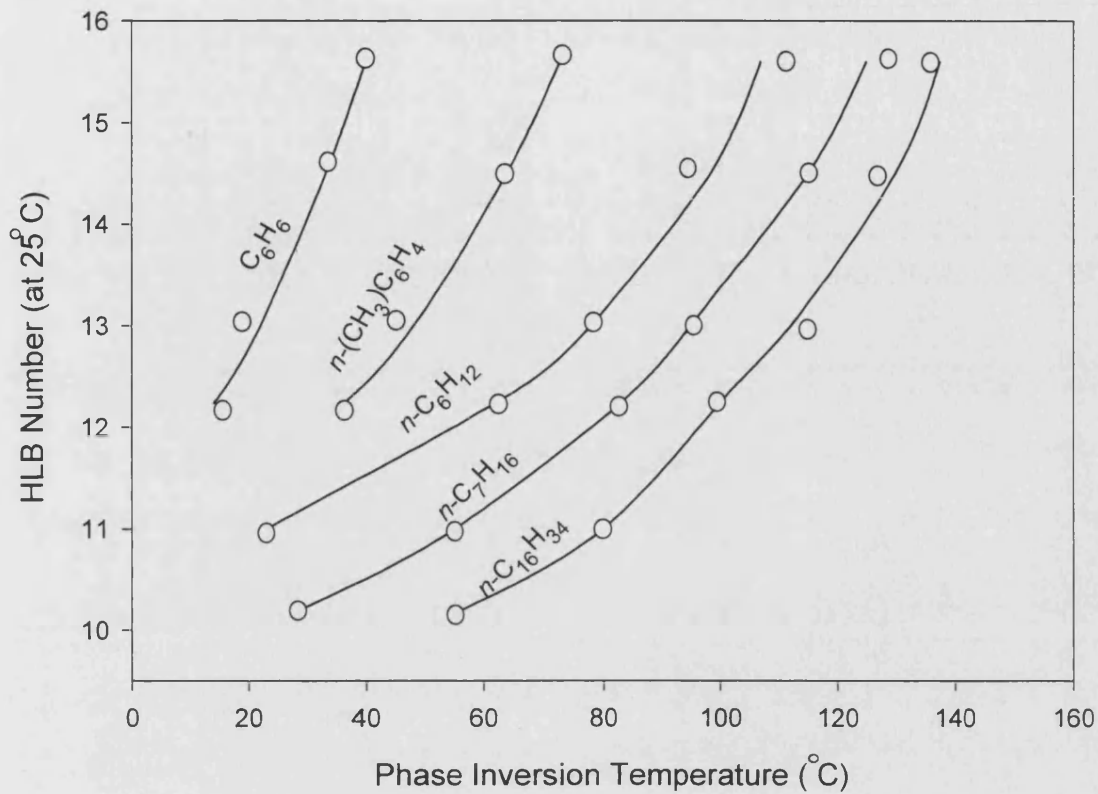
(1) The oil type, (2) Surfactant HLB (hydrophilic chain-length), (3) The effect of phase volume ratio on the PIT and (4) The addition of dissolved solutes

#### 1. Effect of Oil Type on the PIT

Generally, as shown in figure 1.5, the greater the solubility of the surfactant in the oil phase the lower the PIT of the system. Sato and Yuasa [40] have shown that, the greater the affinity of the oil for the surfactant the smaller the reduction in HLB required allowing surfactant to dissolve in oil. Nonpolar oils, such as pure hydrocarbons result in high PIT's whereas polar oils, like for example aromatic hydrocarbons, result in low PITs.

#### 2. Effect of Surfactants on the PIT

Non-ionic surfactants often become increasingly more lipophilic at elevated temperatures. The level of hydration of polyoxythelene chains is decreased by increasing temp and thus HLB, until a critical temp (the cloud point) where the



**Figure 1.5**

The correlation between surfactant HLB number and the PIT in various oil/water (1:1) emulsions stabilized with non-ionic surfactant at concentration of 1.5% w/w (adapted from Ref. 41). The HLB value of the surfactant was changed by increasing the number of ethoxy residues in the surfactant molecule.

surfactant is no longer soluble in water. Shinoda <sup>[41]</sup> has shown that, for a number of different oils, the HLB is a function of temperature (see figure 1.5). HLB numbers are normally assigned to surfactants by measuring the PIT of an emulsion containing the surfactant and checking this against a PIT vs. HLB calibration curve. Shinoda and Aria <sup>[42]</sup> correlated surfactant HLB and PIT for a range of commercial NPE surfactants with cyclohexane. Generally, HLB numbers derived from PIT measurements differ from formula values by <2 HLB numbers. It can be seen from figure 1.5 that, for any oil, the higher the HLB of a surfactant the higher the PIT. Shinoda and Friberg <sup>[31]</sup> also found that the solubility of a nonionic surfactant in particular oil was inversely proportional to the PIT. Graciaa *et al* <sup>[43]</sup> showed that hydrocarbon chain branching in nonionic surfactant-oil-water systems decreases the PIT and effective HLB. This is attributed to the fact that hydrocarbon branching leads to an increased tendency for the surfactant to partition into oil phase.

### Surfactant mixtures

The efficiency of a surfactant in emulsifying and stabilizing a liquid-liquid system is a function of the relative degrees of interactions of the various portions of the surfactant molecule with both the oil phase and the water phase. The HLB of an emulsion system, in which a mixture of surfactants is used (HLB mix), has been generally assumed to be an algebraic mean HLB of the individual surfactants:

$$HLB_{mix} = f_A HLB_A + (1 - f_A) HLB_B$$

Where  $f_A$  is the weight fraction of surfactant A and  $f_B$  is the weight fraction of surfactant B ( $1 - f_A$ )

More stable emulsions are produced when a blend of surfactants is used <sup>[31]</sup>. PIT data indicate that when the difference of HLB of the surfactants in a blend is small, the surfactant mixture HLB is approximately that of the weight average HLB. However, this was not the case for blends with large differences in HLB.

### 3. The effect of Phase volume ratio on the PIT

The effect of the phase ratio of emulsions on the PIT depends on the kind of oil, the concentration of surfactant and the distribution of the polyoxyethylene chain lengths in the surfactant. Shinoda and Friberg <sup>[31]</sup> showed that the PIT does not vary

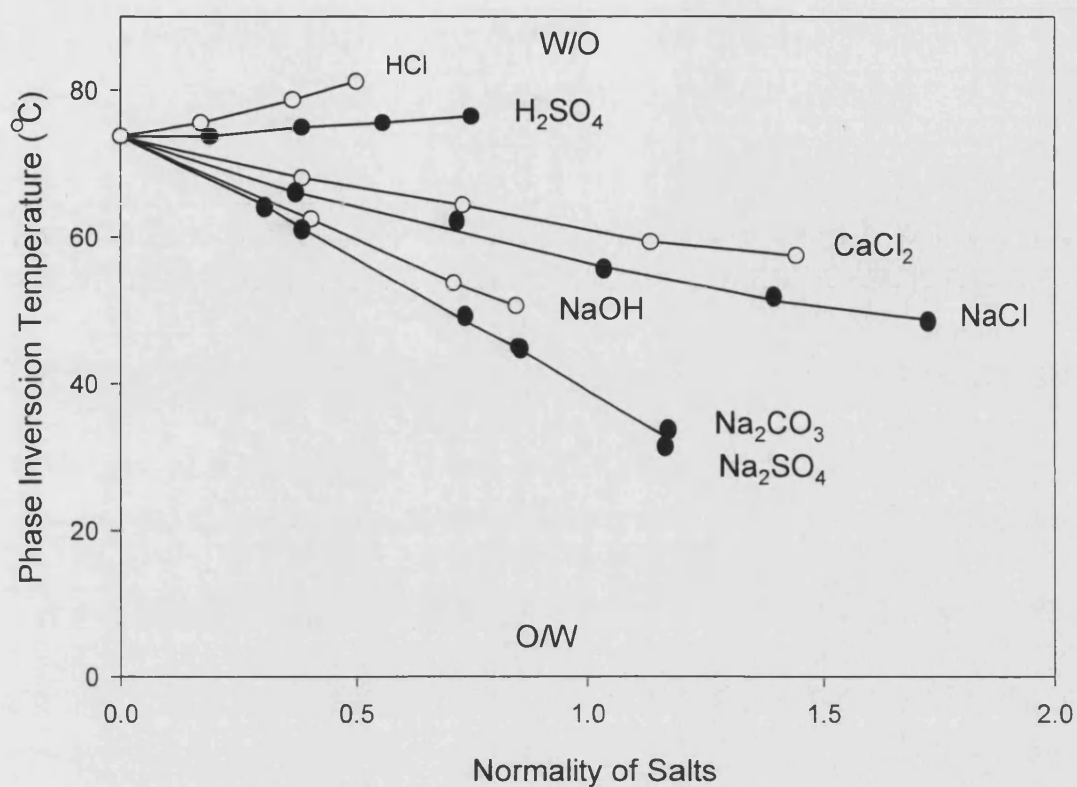
significantly with the phase ratio in non-polar oil systems with surfactant concentration  $>5\%$  wt, but it changes in solutions containing surfactants at concentrations  $<3\%$ . This has been attributed to the fact that the saturation concentrations of nonionic homologues surfactants in water are very small, but those in hydrocarbons are much larger and depend on the ethylene oxide chain length. Lipophilic homologues (shorter chain lengths) dissolve better than hydrophilic homologues in the oil phase. Hence, there will be a selection of more hydrophilic surfactant to be adsorbed at the oil/water interface. This effect is amplified in dilute solutions and when the volume fraction of oil is large. However, in the case of aromatic hydrocarbons and polar oils, Shinoda and Friberg have shown that the phase volume ratio has a marked effect on the PIT

#### **4. The effect of additives on the PIT**

Although the exact mechanism by which various additives affect the PIT is not fully understood, their presence in nSOW systems has been shown to affect the PIT. The phase inversion temperature varies with the amount and chemical type of additives in the water phase. Shinoda and Takeda <sup>[44]</sup> showed that inorganic salts can affect the PIT more strongly than their parent acids. As seen in figure 1.6, the salt depresses all phase boundary temperatures, and hence PIT, in a linear fashion across the whole diagram. This might be due to the fact that some salts depress the PIT by reducing the surfactant HLB and thus its aqueous solubility. However acids increase the PIT and hence the temperature required to achieve maximal O/W or W/O solubilisation. This is comparable to the salting in and salting out phenomena used in crystal purification processes. Shinoda and Friberg <sup>[31]</sup> have shown that the effect of fatty acids and alcohols on the PIT for 1:1 volume ratio paraffin-water systems was independent of the chain length of the acid or alcohol. They have also shown that the more polar the dissolved solute, the greater the reduction in PIT.

#### **1.4. Applications of Microemulsions**

Microemulsions and reverse micellar systems are already applied in certain industrial fields. In the following, certain processes and applications are outlined briefly in order



**Figure 1.6**

The effect of added salts, acids and alkali on the phase inversion temperature (PIT) of cyclohexane/water emulsion (1:1 volume ratio) containing 3% w/w of the nonionic surfactant  $C_9H_{19}C_6H_4O(CH_2CH_2O)_{9.7}H$  in water (adapted from Ref. 44).

to demonstrate their significance and potential. However, pharmaceutical applications of microemulsions, mainly oral drug delivery, will be dealt with in more details as it is the basis of this work.

#### **1.4.1. Enhanced Oil Recovery (EOR)**

Various potential techniques are already applied today in pilot experiments for EOR such as, flooding with surfactants, microemulsions and microemulsion-polymer systems. The method is based on the very low interfacial tensions and good wetting properties of these systems. It considerably enhances the mobilization of oil droplets or oil ganglions entrapped in the rock pores. The interfacial tension ( $\sigma$ ) between crude oil and water is typically  $50 \text{ mNm}^{-1}$ , whereas values of  $10^{-4}$  to  $10^{-6} \text{ mNm}^{-1}$  can be obtained in a suitable microemulsion. This technique implies that an oil reservoir is flooded with surfactant solution and then an external temperature is applied. The interfacial tension between the surfactant solution and the oil decreases with rising temperature. As soon as  $\sigma$  is decreased below a certain limit, a steep increase in oil recovery is observed. Nevertheless, the complete mechanism is not yet understood, as not only the interfacial tension between water and oil is of significance for enhanced oil recovery, but also the coalescence of oil ganglion, emulsification processes and interactions with polymers added to the systems in order to adjust the viscosity. This technique of oil recovery has already been studied intensively due to its high economic significance <sup>[45]</sup>.

#### **1.4.2. Liquid-Liquid Extraction**

Liquid-liquid extraction is mainly of industrial significance for the extraction of metals from ores with low metal contents. The mineral substances are dissolved in a strongly acidic or strongly alkaline aqueous medium from which they are then recovered by two-phase extraction using a specific extractant. These extractants are generally large organic molecules which can be dissolved in the oil-rich domains of the microemulsions. The metal ions, on the other hand, are found in the water-rich domains. Since the interfacial area in microemulsion is much larger than in

conventional stirred two-phase systems, extraction rates can be achieved exceeding those in conventional two-phase systems by a factor of 10 to 100 <sup>[46]</sup>.

### **1.4.3. Lubricants and Cutting Oils**

These liquids very often contain surfactants and water as additives, i.e. microemulsions or reverse micellar solutions are formed with certain compositions. This has two effects: the surfactants cause corrosion inhibition and the increased water content compared to pure oils leads to higher heat capacity. Corrosion inhibition is based, on the one hand, on solubilization and thus on the inclusion of corrosive agents, which can then no longer react with the metal surface. On the other hand, the metal surface is protected by the adsorbed hydrophobic surfactant film. Because of their thermodynamic stability, Microemulsions have succeeded over unstable emulsion systems in this field. The first composition of such a system was patented in 1930 <sup>[47]</sup>.

### **1.4.4. Chemical Reactions in Microemulsions**

Chemists in many fields of activity have studied chemical reactions microemulsions <sup>[48]</sup>. In one of the first studies entitled “Catalysis in Water Pools” Menger described in 1973 the accelerated hydrolysis of esters in w/o microemulsions <sup>[49]</sup>. A large number of publications are available describing organic, inorganic and biochemical reactions in microemulsions.

#### **1.4.4.1. Nanoparticles Preparation**

Discrete nanoparticles with controlled chemical composition and size distribution are readily synthesized using reverse micelles and microemulsions as confined reaction media <sup>[50]</sup>. Yet their assembly into well-defined superstructures amenable to practical use remains a difficult and demanding task. Generally, two microemulsions of the same composition, but containing different reactants in the aqueous fraction, are mixed and the precipitate is then isolated. This technique uses w/o microemulsion droplets as a template for particle growth <sup>[51]</sup> whereby water can be regarded as microreactors. Mei Li et al <sup>[52]</sup> reported that the interfacial activity of reverse micelles



and microemulsions can be exploited to couple nanoparticles synthesis and self-assembly over range of length scales to produce materials with complex organization arising from the interdigitation of surfactant molecules attached to specific nanoparticle crystal faces. Robinson et al <sup>[53]</sup> studied the formation of cadmium sulfide particles in AOT/water/*n*-heptane systems. Superconducting material YBa<sub>2</sub>Cu<sub>3</sub>O<sub>7</sub> was synthesized by microemulsion precipitation, particles of 10 folds lower than the smallest particles formed by bulk solution precipitation was achieved <sup>[54]</sup>. Particle synthesis in microemulsions is a promising technique yet the mechanism of particle formation is not fully established. However, certain factors of influence can be specified and general trends described. Osseo-Asare <sup>[55]</sup> described five aspects to be taken into consideration for particle synthesis in microemulsions: phase behaviour and solubilization, average concentration of the reacting species in the aqueous domains, intramicellar interactions, water/surfactant ratio and structure and properties of the solubilizing water, and dynamic behavior of the microemulsions.

#### 1.4.4.2. Biochemical Reactions

Biotechnological applications of microemulsions have received a great deal of attention since the discovery that enzymes could be solubilized in the droplet cores of W/O microemulsion droplets with retention of activity and stability. The catalytic behavior of Phospholipase A<sub>2</sub> <sup>[56]</sup> and also a large number of enzymes have been studied after being solubilised in W/O microemulsions as reverse micelles. If hydrophobic substrates are added to the enzyme containing microemulsion, it can be observed that the enzyme-dissolved in the aqueous domains converts the substrate dissolved in hydrocarbon phase. Enzyme-catalyzed syntheses with water-insoluble substrates in microemulsions can be carried out by selecting the components for preparing the microemulsions so that no inhibition or denaturation of the enzyme occurs <sup>[57]</sup>. A study of an enzyme-catalyzed reaction including enzyme recovery by extraction from the reaction solution is described by Larsson <sup>[58]</sup>. He has shown that reaction control, product isolation, and enzyme recovery from the microemulsion can be achieved once the phase diagram of the system is established. The isolation of proteins from aqueous solutions by liquid-liquid extraction with a microemulsion is a mature technique <sup>[59]</sup>. By varying the pH value, ionic strength or temperature, the distribution coefficient of a protein can be adjusted between a w/o microemulsion and

the coexisting aqueous phase so that the protein can be absorbed in or released from the microemulsion <sup>[60]</sup>. Large scale recovery of industrial enzymes, proteins and metal/metal ion extractions has been demonstrated using a continuous reactor system. In the forward extraction step the pH is adjusted in the aqueous phase (Winsor I) to favor protein uptake into the reverse micellar phase, whilst in the back extraction, the pH of Winsor II is adjusted to favor release of the protein from the reverse micellar phase.

#### 1.4.4.3. Electrochemical and Electrocatalytic Reactions

Electrochemistry often faces the problem of finding a solvent which simultaneously dissolves an organic substrate and has a sufficient amount of conducting electrolyte. The use of o/w microemulsions as the reaction medium offers very interesting new possibilities of electrochemical analysis and catalysis. The electrochemical syntheses of polyparaphenylenes <sup>[61]</sup> or the dehalogenation of vicinal dihalogen compounds <sup>[62]</sup> are new synthesis paths.

#### 1.4.4.4. Polymerizations

Microemulsions can be used in a similar way to produce polymer particles with a better-defined size and structure in both W/O and O/W microemulsion systems. Tetrahydrofurfurylmethacrylate has been polymerized in an AOT based microemulsion to produce monodisperse microlatices <sup>[63]</sup>. Microemulsions in which these polymerizations can be carried out contain higher surfactant concentrations than the formulations of emulsion polymerization. The monodisperse microlatices obtained from microemulsions are in discussion as carriers for pharmaceuticals. Perez-Luna *et al.* <sup>[64]</sup> gave a good survey of polymerizations in microemulsions and demonstrate in detail the experimental procedure using the example of styrene polymerization.

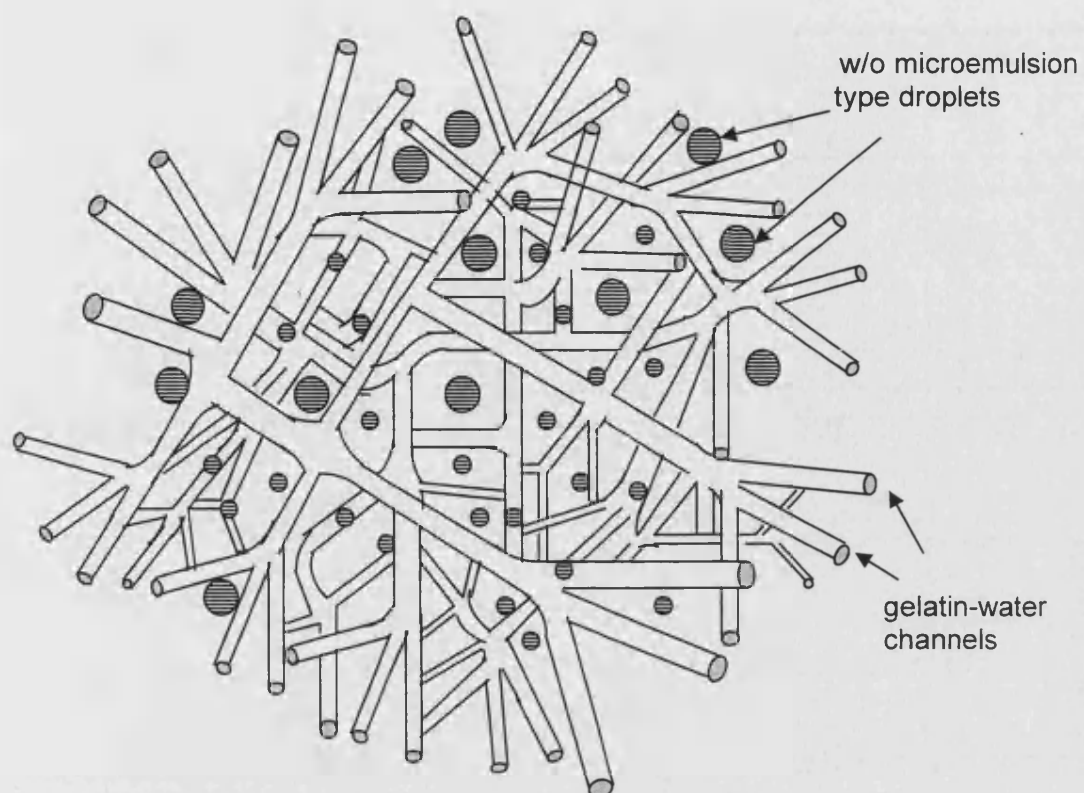
#### 1.4.4.5. Organic Reactions

Organic syntheses are often faced with the problems of reacting water-soluble inorganic reactants with water-insoluble organic reactants. In microemulsions with the aid of amphiphiles, high concentrations of both water soluble and water-insoluble

compounds dissolve simultaneously. This has been recognized in the past 20 years by several groups who used these solutions as reaction media for various organic reactions with reactants such as acids, bases, cyanide, bromide, hypochlorite or permanganate. There are many examples in the literature of organic reactions in microemulsions; alkaline hydrolysis <sup>[49]</sup> using anionic surfactant (AOT) at reactant concentrations of  $10^{-3}$  to  $10^{-2}$  (mol/L), oxidations e.g. of sulfides <sup>[65]</sup> at reactant concentration of 1 mol/L. Generally, the aim is to work with the maximum possible reactant concentrations in conjunction with the minimum possible amphiphile concentrations. After the reaction is complete, the microemulsion is cleaved by changing the temperature so the product and the surfactant will be in two different phases.

#### 1.4.5. Microemulsion-Based Organogels (MBGs)

A number of papers have recently appeared describing the preparation of MBGs by the addition of gelatin to w/o microemulsions. The addition of gelatin to water at sufficiently high concentrations results in the formation of a rigid gel matrix. Gels of similar strength may be easily formed on addition of gelatin to w/o microemulsion. Atkinson <sup>[66, 67]</sup> and co-workers have proposed a structure based on small-angle neutron scattering data which involves an extensive network of surfactant-coated, gelatin- containing conducting aqueous channels coexisting with microemulsion-type droplets. This proposed structure for MBGs is given in figure 1.7. In practical terms, the preparation of MBGs is straightforward. The parent microemulsion is incubated at 50°C and added to a solution of gelatin in water at the same temperature. The resulting mixture is vigorously shaken and then allowed to cool to room temperature when an optically transparent single phase gel is formed. The rigidity of MBGs is controlled in the main by the added amount of gelatin. The MBGs are prepared using different varieties of surfactants and oils including AOT/isooctane <sup>[68]</sup> and Tween 80/IPM (isopropyl myristate) <sup>[69]</sup>. The organogels are optically clear and thermoreversible, and have been suggested as novel delivery vehicles for drugs and antigens <sup>[70]</sup>. Recently, lipases have been immobilized in MBGs and the synthesis of a wide variety of structurally different esters has been achieved on a preparative scale.



**Figure 1.7**

Structure of microemulsion-based organogels (MBGs); aqueous (w/o) microemulsion droplets in equilibrium with a network of rods of gelatin and water surrounded by the surfactant AOT (adapted from Ref. 66).

### 1.4.6. Pharmaceutical Applications of Microemulsion Systems

#### 1.4.6.1. Pharmaceutically Acceptable Excipients for Microemulsions

Although several microemulsion systems have been described in the literature, the challenge for the pharmaceutical formulator is to predict which oil(s) and surfactant(s) to select for a particular application.

Pharmaceutically acceptable oils tend to be more polar and of much higher molecular weight than the more commonly used aliphatic or aromatic oils. Medium chain glycerides derived from coconut oil (such as Miglyol 812, a mixture of C<sub>8</sub>/C<sub>10</sub> triglycerides) are particularly attractive for formulating orally active microemulsions for many reasons:

1. They are stable food grade products and generally recognized as safe (GRAS) by the Food and Drug Administration agency <sup>[71]</sup>.
2. Microemulsions incorporating these excipients can be formulated at ambient temperature over a wide range of compositions <sup>[72]</sup>.
3. Medium-chain glycerides (mono-, di-, and triglycerides) are reported to improve the intestinal absorption of co-formulated drugs <sup>[73, 74]</sup>.
4. They could be easily formulated and presented in soft gelatin capsules, or recently in sealed hard gelatin capsules.

Recently polyglycolized glycerides (PGG) with varying fatty acid and polyethylene glycol (PEG) chain lengths and thus varying HLB, in combination with vegetable oils have been used to solubilize poorly water-soluble drugs and improve their bioavailability <sup>[14]</sup>.

Ionic surfactants are frequently used for the production of microemulsions; they however have only a very limited use for pharmaceutical purposes <sup>[75]</sup>, where the surfactants of choice are either nonionic or zwitterionic. This is because of the lower toxicity of nonionic and zwitterionic surfactants, in particular membrane toxicity, and their greater stability towards the changes in ionic strength and pH that are to be encountered in the biological environment <sup>[76]</sup>.

The nonionic surfactants studied include; polyoxyethylene hydrogenated castor oil, sucrose esters, polyoxyethylene sorbitan fatty acid esters, polyoxyethylene n-alkyl ethers, polyglycerol fatty acid esters and polyoxyethylene monoglyceride. A number of nonionic surfactants such as the polyoxyethylene n-alkyl ethers <sup>[75]</sup>, and

polyoxyethylene hydrogenated castor oil <sup>[77]</sup> do not require a cosurfactant for the formation of microemulsion. Sucrose esters and polyglycerol fatty acid esters, on the other hand, are suitable for pharmaceutical purposes yet the presence of an alcohol cosurfactant is required to produce microemulsions <sup>[78, 79]</sup>.

The use of a cosurfactant is generally considered to be undesirable in pharmaceutical applications <sup>[75]</sup>. The majority of studies have used medium chain length alcohols as the cosurfactant of choice. There are significant toxicity and irritancy issues with these materials, which preclude their use in pharmaceutical formulations. The aqueous solubility of cosurfactants in o/w microemulsion systems is often higher than that of the principal surfactant. Consequently, when the o/w microemulsion is diluted, the cosurfactant partitions more strongly to the aqueous phase. As a result, depletion of the cosurfactant at the oil/water interface occurs, thereby destabilizing the microemulsion droplet. As a consequence, workers have examined alternative cosurfactants such as medium chain mono- and diglycerides <sup>[74]</sup>, alkan-1,2 diols <sup>[80]</sup>, ethanol and polyhydric alcohols such as sorbitol and sucrose <sup>[81, 82]</sup>.

Lecithin-based microemulsions, both o/w and w/o, have recently been considered as alternative drug delivery systems that avoid problems of toxicity associated some of the nonionic surfactants <sup>[83]</sup>. However, since lecithin is too hydrophobic (HLB = 4) and has a tendency to form lamellar liquid crystalline phases, short-chain alcohols are often included to alter the HLB and aid emulsification by destabilizing the liquid-crystalline phases <sup>[84]</sup>. Therefore, phospholipid microemulsions have primarily been used for topical drug delivery <sup>[83]</sup>.

The choice of emulsifiers is determined by the average HLB requirement of the proposed microemulsion (see section 1.3.3). Water-in-oil microemulsions are formed using emulsifiers within the HLB range of 3-8 while o/w microemulsions are formed within the range of 8-18. Some of the oils and surfactants used to formulate microemulsions for oral drug delivery along with their HLB values are listed in table 1.2.

#### 1.4.6.2. Drug Incorporation into Microemulsions

In spite of a large number of therapeutic agents of varying physicochemical properties being incorporated into microemulsions, very little work has examined the influence of drugs on the phase behavior and stability of the microemulsion. Phase diagrams

**Table 1.2: Some of the common excipients used to formulate lipid microemulsions for oral delivery** (modified from Ref. 76)

Excipient	HLB Value	Chemical Definition	Manufacturer
Arlacel 80	4.3	sorbitan oleate	ICI Americans
Arlacel 186	2.8	monoolein:propylene glycol (90:10)	ICI Americans
Capmul MCM	5.5-6	C <sub>8</sub> /C <sub>10</sub> mono-/diglycerides from coconut oil	Abetic
Captex 200	oil	C <sub>8</sub> /C <sub>10</sub> diesters of propylene glycol from coconut oil	Abetic
Captex 355	oil	C <sub>8</sub> /C <sub>10</sub> triglycerides from coconut oil	Abetic
Centrophase 31	4	liquid lecithin	Central Soya
Cremophor EL	3.5	35-polyoxyethylene castor oil	BASF
Cremophor RH 40	14-16	40-polyoxyethylene hydrogenated castor oil	BASF
Labrafac CM 10	10	C <sub>8</sub> /C <sub>10</sub> polyglycolized glycerides from coconut oil	Gattefosse
Labrafil M 1944	3-4	oleic acid (C <sub>18:1</sub> ) polyglycolized glycerides from apricot kernel oil	Gattefosse
Labrafil M 2125	3-4	linoleic acid (C <sub>18:2</sub> ) polyglycolized glycerides from apricot kernel oil	Gattefosse
Labrasol	14	C <sub>8</sub> /C <sub>10</sub> polyglycolized glycerides from coconut oil	Gattefosse
Miglyol 812	oil	C <sub>8</sub> /C <sub>10</sub> triglycerides from coconut oil	Huls
Myvacet	oil	distilled acetylated monoglycerides	Eastman chemicals
Myverol 18-92	3.7	distilled sunflower oil monoglycerides	Eastman chemicals
Soyabean	oil	oleic (25%) and linoleic (54%) triglyceride	Croda
Tagat To	11.3	polyoxyethylene (25) glycerol trioleate	Goldschmidt Chem.
Tween 80	15	polyoxyethylene (20) sorbitan oleate	BASF

should be constructed in the presence of a particular drug, particularly if the drug is surface active and thus expected to affect significantly the microemulsion region. Also in cases where lipophilic drugs are incorporated into o/w microemulsions, such drugs frequently act as oils themselves and therefore influence phase behavior. However, low levels of potent water-soluble drugs such as, peptides added to w/o microemulsions probably will not have a significant effect on initial phase studies.

The amount of drug incorporated into a given microemulsion is dependent on its relative solubility in the various component systems, particularly on its oil/water partition coefficient. Pre-formulation data that includes aqueous solubility, as well as solubility in selected microemulsion excipients should precede any microemulsion formulation work.

In selecting an appropriate type of microemulsion for a particular drug certain physicochemical data are required, in particular, aqueous and/or oil solubility or even better partition coefficients <sup>[73]</sup> along with, for all routes of delivery except intravenous, its *in vitro* membrane permeability <sup>[76]</sup>. The drug classification by Amidon *et al.* <sup>[85]</sup> based on drug solubility and intestinal permeability best describes some of the factors controlling the drug dissolution and absorption process. This biopharmaceutical drug classification to address drug dissolution and absorption for each of the four drug classes along with the recommended microemulsion systems is presented in table 1.3

#### 1.4.6.3. Water-in-Oil Microemulsions

Water-in-oil microemulsions have been designed to overcome metabolic and physical barriers to water soluble drug molecules, particularly peptides and proteins. One of the reasons why microemulsions in general are attractive for the delivery of labile proteins and peptides is that they do not require high temperatures and/or homogenization for their preparation <sup>[76]</sup>. Water-in-oil microemulsions improve the oral bioavailability of drugs by protecting these sensitive drugs from the harsh proteolytic environment of intestine and also they enhance their intestinal absorption *per sec* <sup>[76]</sup>.

Water-in-oil microemulsions as an oral drug delivery enhanced the absorption of insulin, calcitonin and growth hormone <sup>[86]</sup>. Ritschel and co-workers <sup>[87]</sup> showed that



**Table 1. 3: Potential microemulsion systems for drugs based on physicochemical and biological properties** (modified from Ref. 76 and 85).

<i>Drug Class</i>	<i>Aqueous Solubility</i>	<i>Membrane Permeability</i>	<i>Potential Microemulsion System</i>	<i>Possible advantages</i>
Class I	High	High	w/o or o/w	Protection against enzymatic and hydrolytic degradation
Class II	Low	High	o/w	Improved solubilization and dissolution, enhanced bioavailability
Class III	High	Low	w/o	Protection against enzymatic and hydrolytic degradation, enhanced bioavailability
Class IV	Low	Low	o/w	Improved solubilization and dissolution, enhanced bioavailability

\*\* Compounds are considered to have low aqueous solubility ( $S_0$ ) and apparent permeability ( $P_{app}$ ) at  $S_0 < 100\mu\text{g/ml}$  or at  $P_{app} < 2 \times 10^{-6} \text{ cm/s}$ , respectively.

**Table 1.4: Composition of Neoral® compared with Sandimmun®** (adapted from Ref. 96)

<i>Neoral®</i>	<i>Amount</i>	<i>Sandimmun®</i>	<i>Amount</i>
Cyclosporin	100mg	Cyclosporin	100mg
1,2 propylene glycol	75mg	Ethanol	100mg
Ethanol	150mg	Maize oil	416mg
Partial glycerol transesterified corn oil	345mg	Transesterified triglyceride and Polyalkaline polyol (Labrafil M2126)	300mg
Cremophor RH 40	405mg		
Total amount per dose	1075mg	Total amount per dose	916mg

absorption of vasopressin in rats from ligated small intestinal segments was increased by about two-fold when the peptide was formulated in the microemulsion as compared to an aqueous solution. Water-in-oil microemulsions encapsulating water-soluble biologically-active materials, such as peptides, have been reported by Owen *et al.* [88]. It is obvious that upon oral administration a w/o microemulsion undergoes a considerable dilution and, as a consequence, a phase inversion to an o/w microemulsion releasing the encapsulated water-soluble molecule.

Both o/w and w/o microemulsion formulations for topical or transdermal application have been investigated by various workers. It was shown that it is possible to formulate an effective topical preparation of the local anesthetic pentacaine chloride [89] in a w/o microemulsion. Skin permeation of felodipine, a calcium antagonist, has also been investigated from o/w microemulsions stabilized by a surfactant mixture containing Tween 20 and taurodeoxycholate [90]; Isopropyl meristate (IPM) was used as the oil phase with benzyl alcohol as a cosurfactant. Water-in-oil microemulsion formed from diclofenac dimethylamine and phospholipids have been examined as transdermal delivery vehicles [91]. The formulation was based on a combination of Tween 80 and span 20 with IPM. Diclofenac dimethylamine was released at a much faster rate from the microemulsion formulations than an aqueous solution of the drug or from gels and liposomes.

Water-in-oil microemulsions have been shown to form thermodynamically stable gels, known as microemulsion-based gels (MBG, see section 1.4.5) upon the addition of gelatin. These oil-based, electrically conducting gels have been recently successfully developed for the transdermal delivery of water-soluble drugs using a technique called iontophoresis. A small direct electric current is applied to the skin in order to drive small charged molecules into the body and into the blood circulation [92].

#### 1.4.6.4. SMEDDS & Oil-in-Water Microemulsions

Self-Micro-emulsified drug delivery system (SMEDDS) is the name given to a drug vehicle consisting of oil or modified oils, surfactant and co-surfactant mixtures, solid or liquid at ambient temperature, which emulsifies spontaneously when mixed with water at 37°C under gentle agitation [93]. This system is able to form a microemulsion

of droplets with diameters between 5 and 140nm when they are in contact with the aqueous media such as gastro intestinal fluids, so they potentially increase most drug dissolution and bioavailability. SMEDDS performance depends on: firstly, the mixtures ability to emulsify when diluted in a large quantity of water. Secondly, the HLB value which is related to the solubilizing behavior of the preparation <sup>[94]</sup>. Drug delivery advantages offered by microemulsions include: improved drug solubilisation and protection against enzymatic hydrolysis, as well as the potential for enhanced absorption afforded <sup>[95]</sup>.

The most notable example of SMEDDS is the oral delivery of cyclosporin A as a commercial formulation Neoral<sup>®</sup>. Cyclosporin A is a cyclic undecapeptide used as an immunosuppressant in transplantation surgery, and in contrast to most peptide drugs is hydrophobic. The original Sandimmune<sup>®</sup> formulation was based on a solution of cyclosporin A in vegetable oil (table 1.4). It formed, after oral administration, a crude o/w mixture which had to be emulsified by the bile salts present in the small intestine in order to initiate the digestion of the oil droplets and therefore the release of the cyclosporin. Although the co-administration of triglyceride with the cyclosporin A improved its bioavailability, there was considerable pharmacodynamic inter- and intra-patient variability <sup>[97]</sup>, bioavailabilities as low as 1% and as high as 89% have been reported <sup>[98]</sup>. This variation can be attributed to factors such as food intake and the mechanism of uptake *in vivo* which is considered to be related to the lipolysis of the triglyceride. The new Sandimmune Neoral<sup>®</sup> formulation (table 1.4) is described as a microemulsion pre-concentrate which apparently produces an oil-in-water microemulsion on contact with the gastrointestinal fluids without the requirement for the involvement of endogenous compounds such as bile salts, and consequently the bioavailability and reproducibility of delivery is greatly enhanced. The available data has been reviewed by Noble and Markham who confirm the conclusion that Neoral<sup>®</sup> offers more predictable and more extensive drug absorption than the standard Sandimmune<sup>®</sup> formulation <sup>[98]</sup>. Almost 2-fold increase in the oral bioavailability of cyclosporin was observed in the case of Neoral<sup>®</sup> compared with the old formulation. Recently, an o/w microemulsion of insulin was gelled using Carb-O-Sil and orally administered in polymer-coated gelatin capsules designed so that release of the insulin would occur mainly in the colon <sup>[99]</sup>. Oil-in-water microemulsions have also been proposed as aqueous-based vehicles for the pulmonary delivery of lipophilic drugs via

nebulization <sup>[100]</sup>. This interest is a consequence of the imminent ban by 2005 on chlorofluorocarbon propellants used in inhalers and the problems experienced with the reformulation of existing drugs in the alternative hydrofluoroalkanes. The formulation of a water-in-HFA propellant microemulsion stabilized by fluorocarbon non-ionic surfactant has been investigated by Patel *et al.* <sup>[101]</sup> for pulmonary delivery. The development and characterization of o/w microemulsions designed for parenteral <sup>[102]</sup> and ocular use has also been reported recently <sup>[103]</sup>.

## 1.5. Classification of Lipid Delivery Systems

A very recent attempt has been made by Pouton <sup>[104]</sup> to classify lipid formulations for the enhancement of bioavailability of hydrophobic drugs. He suggests three main criteria with which lipid formulations can be distinguished:

- a) Does the formulation self-emulsify or remain poorly dispersed in water?
- b) When the formulation makes contact with water are some of the components lost by dissolving in the aqueous phase?
- c) Is the dispersed formulation digestible by lipases?

Answering these proposed questions Pouton has derived three major types of formulations with lipid systems. These types result from blending up to five classes of excipients; ranging from pure triglycerides oils, through mixed glycerides, lipophilic surfactants, hydrophilic surfactants and water soluble co-solvents. Table 1.5 describes the typical properties of the different types of lipid formulations

Type (I): easily digestible formulations which consist of triglycerides and/or mixed glycerides in the absence of surfactant. Type (II): self-emulsifying formulations which comprise triglycerides and/or mixed glycerides, and lipophilic surfactants (HLB<12). Type (III): self-emulsifying systems which comprise triglycerides, mixed glycerides, hydrophilic surfactants (HLB >12), and water-soluble co-solvents.

### 1.5.1. Type I Lipid Formulations

Formulations which comprise drug in solution in triglycerides and/ or mixed glycerides are classified as Type I (Table 1.5). When an appropriate dose of the drug can be dissolved, this type of formulations will be the system to be considered for its

**Table 1.5: Typical properties of Type I, II, IIIA and IIIB lipid formulations** (adapted from Ref. 104)

	Increasing hydrophilic content →			
	Type I	Type II	Type IIIA	Type IIIB
Typical composition (%) Triglycerides or Mixed glycerides	100	40-80	40-80	<20
Surfactants	0	20-60 (HLB<12)	20-40 (HLB>12)	20-50 (HLB>12)
Hydrophilic cosolvents	0	0	0-40	20-50
Particle size of dispersion (nm)	Coarse	100-250	100-250	50-100
Significance of Aqueous dilution	Limited importance	Solvent capacity unaffected	Some loss of solvent capacity	Significant phase changes and potential loss of solvent capacity
Significance of digestibility	Crucial requirement	Not crucial but likely to occur	Not crucial but may be inhibited	Not required and unlikely to happen

simplicity and biocompatibility. If sufficient solvent capacity cannot be achieved using pharmaceutically acceptable mixtures of triglycerides, mixed glyceride or other lipophilic esters, this type will not be an option. Type I formulations on dilution forms coarse O/W macro-emulsion thus, the dispersion of the drug into colloidal solution is likely to be dependent on digestion by lipolysis, a process which takes place mainly in the small intestine. During digestion the drug is solubilised in mixed micelles of bile salts and digestion products and thus high bioavailability can be expected yet without rapid onset of action. If the drug has a low therapeutic index Type I formulation will be more favorable than other types. This type has the advantage that on dilution in aqueous media, the solubilised dose of the drug faces no risk of precipitation. From a clinical and toxicological perspective, Type I formulations may be safer with reference to the generated pharmacokinetic profile and also since they avoid the chronic administration of surfactants.

**Table 1.6 :Selected examples of Type I lipid formulations**

<i>Compound</i>	<i>Lipid</i>	<i>Reference</i>
Triamterene	Peanut oil, oleic acid	105
Cyclosporine	Olive oil	106
Griseofulvin	Corn oil	107
Penclomedine	Mineral oil	108
Prbucol	Liquid paraffin	109
Vitamin E	LCT, MCT	110

### 1.5.2. Type II Lipid Formulations

These systems are defined as self-emulsifying formulations that consist of water insoluble components. Nonionic ester ethoxylate surfactants with HLB of 10-12, such as polysorbate 85 (Tween 85) or polyoxyethylene 25-glyceryl trioleate (Tagat TO) form the core component of Type II to promote emulsification of triglycerides or mixed glycerides, especially medium chains. These surfactants do not form micelles in water, and are described by their manufacturers as water dispersible. Yet, in an

excess of water they form a fine stable emulsion as they interact strongly with water forming liquid crystal phases. Self-emulsification of this system is closely related to the formation of dispersed lamellar liquid crystals on dilution with water. The lipophilic surfactant concentration in Type II systems requires optimization. As the surfactant content in the blend is increased there is a threshold at approximately 25% (w/w) surfactant beyond which self-emulsification occurs <sup>[111]</sup>. At high surfactant concentrations, more than 65%, emulsification is compromised by viscous liquid crystalline gels which form at the oil-water interface. This type of formulation is likely to retain its solvent capacity for the drug after dispersion in aqueous media. Type II systems consisting of medium chain triglycerides and polyoxyethylene - (25) - glyceryl trioleate (Tagat TO) have produced particles as fine as 100-250 nm, depending on the surfactant concentration <sup>[7]</sup>. Self-emulsification of Type II formulations can be further improved; this is reflected in the dramatic reduction of droplet size, by incorporating medium-chain glycerides, particularly C<sub>8</sub>/C<sub>10</sub> mono/diglycerides such as Imwitor 988 which acts as a co-surfactant. Yet, the ratio of medium chain triglycerides to (mono/diglycerides) as well as the surfactant concentration should be optimized. Many type II systems will be digestible unless the surfactant concentration is too high. However, lipolysis of this type of systems is not crucial for the enhancement of bioavailability of lipophilic drugs, though it is likely to occur.

**Table 1.7 : Selected examples of Type II lipid formulations**

<i>Compound</i>	<i>Formulation</i>	<i>Reference</i>
WIN 54954	Neobee-M5(MCT)/Tagat TO	13
CCK <sub>8</sub> receptor antagonist	Labrafil M2125/Tween 80	112
Benzoic acid	Miglyol 812/Tween 85	5
Ro 15-0778	Neobee-N5/Peanut oil	14

### 1.5.3. Type III Lipid Formulations

This type of formulations fulfils the need to improve the solvent capacity of lipid formulations. This is achieved by inclusion of hydrophilic nonionic surfactants and or hydrophilic co-solvents.

- Examples of hydrophilic surfactants (HLB>12): Cremophor RH40, Cremophor EL, Emuline and Tween 80. These surfactants form micellar solution when transferred to the aqueous phase on dispersion.
- Examples of hydrophilic co-solvents: Ethanol, Propylene glycol, Polyethylene glycol, Glycerol, Glycofurol and Transcutol.

The introduction of more hydrophilic components reflects on the mechanism of emulsification of this type of formulations, the droplet size produced and also the lipolysis of these systems. The mechanism of emulsification involves diffusion of the hydrophilic components away from the bulk oil into the aqueous phase, thus 'diffusion and stranding' becomes a predominant mechanism for emulsification which will be discussed in more detail next. These systems are also called self-microemulsifying systems, as optical clarity can be achieved with these formulations. When the surfactant content is high (for example, >30% w/w) or co-solvents are included in addition to surfactants, very fine dispersions (50-100nm) can be produced under conditions of gentle agitation <sup>[113]</sup>. Type III systems have been shown to act independently of bile, which suggests that they are not necessarily digested before the drug is absorbed <sup>[114]</sup>. The purpose for formulating with Type III systems may also be to increase the solvent capacity for drugs with log *P* 2-4. As this type of formulations has water soluble components, there is a tendency to be loss of solvent capacity on dilution in the aqueous phase. The consequence may be that the drug is partially precipitated when the formulation disperses. The risk of precipitation is greater when the formulation contains a higher proportion of hydrophilic components. Pouton has further sub-classified Type III formulations into Type IIIA and Type IIIB addressing the degree of hydrophilicity of this type. Both Type IIIA & B have hydrophilic surfactant and co-solvent yet they differ in the oil content. A typical Type IIIA formulation contains approximately 50-60% triglycerides or mixed glyceride content, whereas Type IIIB has less than 20%. This renders type IIIB the most hydrophilic type of lipid formulation, which is likely to result in a colloidal solution of drug and oil in aqueous micelles. As a result, Type IIIB presents the highest risk of precipitation. It also produces the finest dispersions, due to their high content of water-soluble solubilising agents. An archetypal example of a Type III system is the reformulation of cyclosporine A as Neoral<sup>®</sup> <sup>[104]</sup>. The drug was more available from the Neoral than the earlier 'Sandimmune' formulation, which was a coarsely



emulsifying system <sup>[115]</sup>. This might be due to the fact that the coarse emulsion produced by the Sandimmune formulation could not be reduced to colloidal dimensions because of limited digestion <sup>[111]</sup>.

## 1.6. Mechanisms of Emulsification

### 1.6.1. Emulsification of Type II Lipid Formulations by Interfacial Liquid Crystal Formation (ILCF)

A general mechanism for spontaneous emulsification in nonionic surfactant based on pesticide formulations was proposed by Groves <sup>[116]</sup>. Self-emulsification was suggested to be a result of dynamic formation of liquid crystalline units at the interface promoting further water penetration into the oily phase down aqueous channels. The resulting increase in surface pressure would lead to interfacial disruption and dispersion of the oil as droplets into the bulk aqueous phase <sup>[7]</sup>. As illustrated in figure 1.8a, water initially diffuses into the nonionic surfactant-oil mixture and associates by hydrogen bonding with the surfactant molecules. This will occur until the solubilisation limit is reached close to the interface. Figure 1.8b shows further aqueous penetration which will result in the formation of dispersed lamellar liquid crystal phase ( $LC_A$ ) shown as parallel lines. This material has a loosely associated lamellar lattice structure. As the aqueous penetration proceeds, eventually almost all material close to the interface will be liquid crystalline. The actual amount of the liquid crystals formed depends on the surfactant concentration in the binary mixture. Once these have been developed water continues to penetrate into the oil-surfactant mixture through the aqueous channels in the liquid crystals or gel matrix (figure 1.8c). The rate of this penetration will be dependent on the degree of orientation within the structured phase and the size of the aqueous pores. The increase of the water transport through the aqueous cores is accompanied by an increase of the surface pressure close to the interface. The interfacial instability, in conjunction with a reduced interfacial tension due to the increase in surface pressure aided by the gentle agitation during the emulsification process, causes a rapid expansion of the water – liquid crystal boundary to equalize the pressure, resulting in interfacial disruption and emulsification.

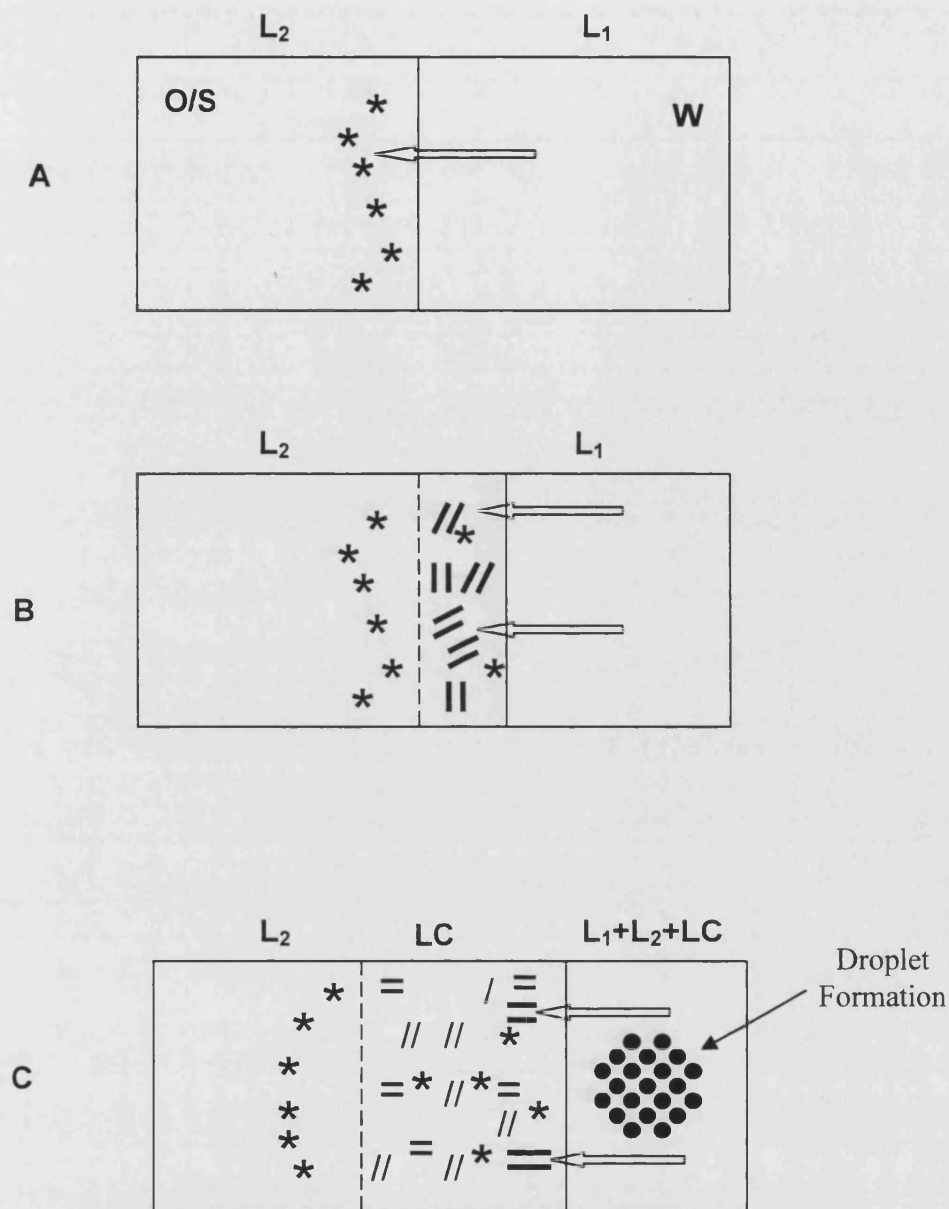


Figure 1.8

Schematic Representation of Self-Emulsification Mechanism proposed by Wakerly *et al.* <sup>[7]</sup> (A) Water Penetration (B) Formation of Liquid Crystals (C) Disruption and Emulsification.

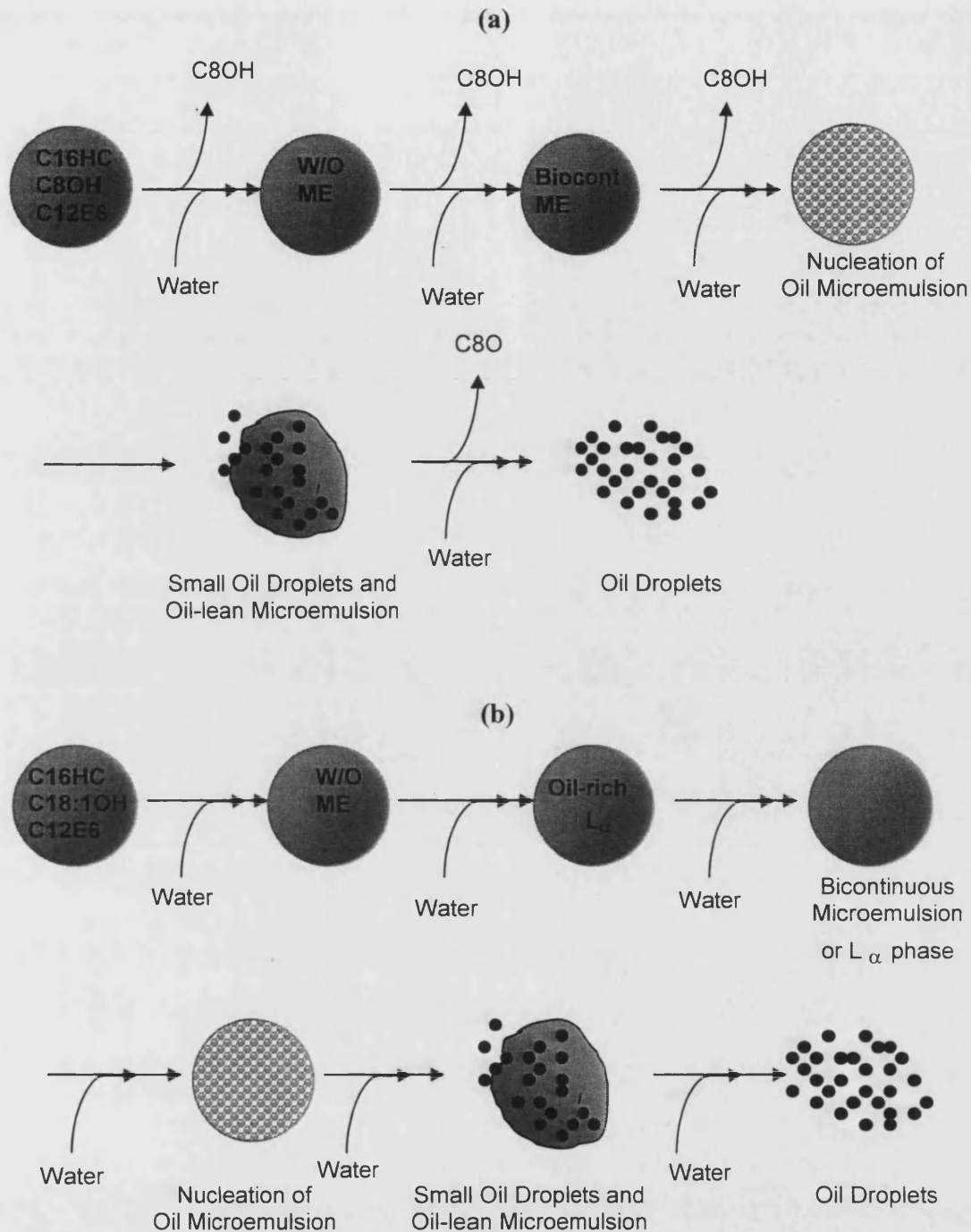
Surfactant molecules will continue to diffuse to the interface to replenish the liquid crystal layer due to the induced diffusion gradient.

### 1.6.2. Emulsification of Type III Lipid systems by 'Diffusion and Stranding' Mechanism.

This mechanism was first proposed by Gurwistch <sup>[117]</sup> as an alternative to spontaneous emulsification, which would take place without interfacial turbulence. It deals with emulsification resulting from differential solubilities of a third component in the oil and water phases. Generally, systems which do not contain surfactants, for example the ethanol/toluene/water system examined by Haydon <sup>[118]</sup>, are quoted as examples of this mechanism. In this approach the dissolved third component (ethanol) has a considerable solubility in the receptor phase (water) as well as the phase in which it is initially dissolved (toluene). The dissolved material therefore diffuses across the interface from one phase to the other. In so doing it can carry with it some of the solvent phase (toluene) into the receptor phase (water). Once this has occurred the oil phase, having a low solubility in water, forms droplets which increase in size as more oil transported. These droplets slowly move away from the oil-water interface due to the kinetic energy imparted by the mass transfer process.

In the multi-component systems examined by Gilbert <sup>[119]</sup>, surfactants are initially contained in the oily phase. It is proposed that water penetrates into the oil phase prior to exudation of oily jets from the interface subsequently, the surfactant becomes more hydrated. This will increase the surfactant solubility in the aqueous phase and render it less soluble in the oil phase. As a result the surfactant diffuses into the aqueous phase carrying oily components with it. This is an example of the diffusion and stranding mechanism specific to systems containing hydrophilic surfactants.

Rang *et al.* recently investigated the spontaneous emulsification of *n*-hexadecane/*n*-octanol( $C_8OH$ )/hexaethyleneglycolmono-*n*-dodecyl ether ( $C_{12}E_6$ ) <sup>[120]</sup> and *n*-hexadecane/oleylalcohol( $C_{18:1}OH$ )/ $C_{12}E_6$  <sup>[121]</sup> mixtures. In the first system as shown in figure 1.9a, diffusion of water into the oil phase converted it to a microemulsion that subsequently became supersaturated in oil due to diffusion of octanol into the water. As a result small oil droplets (of order 1  $\mu m$ ) nucleated spontaneously from the microemulsion. Further diffusion of octanol out of the now oil-lean microemulsion phase made it ever more hydrophilic so that it eventually became miscible with water.



**Figure 1.9**

Schematic diagram showing spontaneous emulsification process for drops of **(a)** *n*-hexadecane/*n*-octanol(C8OH)/C12E6 containing water or **(b)** *n*-hexadecane/oleyl alcohol/C12E6 containing water (adapted from Ref. 121).

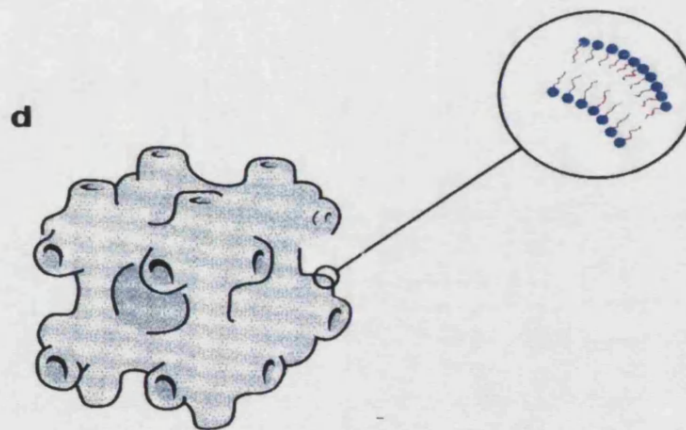
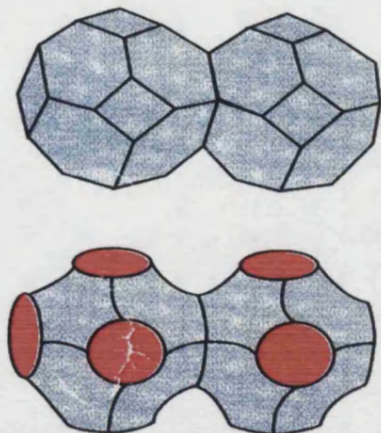
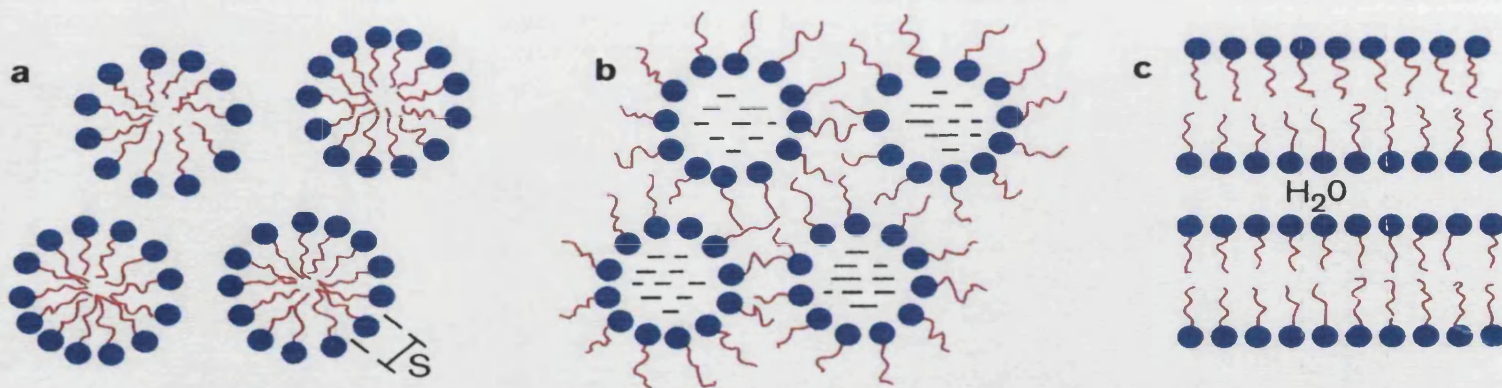
Thus, for some optimal range of initial drop compositions, an emulsion of small oil droplets in water resulted. It is noteworthy that no liquid crystalline phase was involved in first system. However, using the longer chain alcohol oleylalcohol( $C_{18:1}OH$ ) as in the second system, the injected oil drop is completely converted to  $L_\alpha$  (lamellar liquid crystal) and also to microemulsion. Thus, any oil present in the final state must have been nucleated from one of these phases;  $L_\alpha$  or microemulsion. The process is shown schematically in figure 1.9b.

## 1.7. Lipid-Water Interaction & Liquid Crystalline Phases 1

Liquid crystals are ordered structures, without the stiffness of crystalline solids. They contain a polar, a non-polar and an amphiphilic component and are thermodynamically stable, i.e. they self-organise spontaneously. In theory, based on mathematical symmetry calculations, eighteen possible structures can be formed <sup>[122, 123]</sup>, many of which have been identified by Rosevear <sup>[124]</sup>. However, in two component systems only three major types of liquid crystal are formed, these being classified by the number of dimensions in which the crystal structure pattern is repeated. The lamellar, hexagonal and cubic forms are the most likely to be found using lipid based components.

### 1.7.1. Lamellar Liquid- Crystalline Phase ( $L_\alpha$ )

More commonly in lipids a liquid crystalline phase is formed in the presence of water, as shown in figure 1.10. Above a critical hydrocarbon-chain melting temperature, water penetrates the polar region and a lamellar lipid-water structure ( $L_\alpha$ ) is formed with water layers alternating with lipid bilayers (figure 1.10c). The surfactant molecules are oriented in the bilayer such that the lipophilic hydrocarbon chains of the molecule are intermeshed in a central sandwich core with the hydrophilic portions localized on both sides. The lamellar structure (also known as the neat phase) has a one dimensional periodicity and is capable of liquid flow in two directions by means of sliding over one another. Due to the relative ease of flow the viscosity of this phase is normally the lowest of the liquid crystal classes.



**Figure 1.10**

Polymorphic phases available to hydrated liquid crystalline lipids  
(a) Hexagonal  $H_I$ , (b) Hexagonal  $H_{II}$ , (c) Lamellar ( $La$ ) and (d) Cubic

Illustration adapted from The Lipid Handbook (Ref. 125).

### 1.7.2. Hexagonal Phase ( $H_I$ & $H_{II}$ )

The hexagonal or middle mesophase is periodic in two dimensions. The two hexagonal structures shown in figure 1.10 (a, b) are termed  $H_I$  and  $H_{II}$  respectively. The  $H_I$  phase consists of infinite cylinders of lipid molecules with their polar groups oriented toward the outer (continuous) water phase, and the surfactant of hydrocarbon chains filling out the core of the cylinders. Because of the continuous water phase,  $H_I$  can be diluted indefinitely forming spherical o/w micelles. The  $H_{II}$  phase is the inverse of  $H_I$ , i.e. water forms cylinders in a continuous medium of surfactant molecules with the polar groups oriented toward the water phase and the hydrocarbon chains filling out the exterior between the cylinders. Unlike  $H_I$  phase,  $H_{II}$  has a limited swelling capacity, and it can only accommodate approximately 40% water in the cylindrical aggregates. The lamellar and hexagonal phases can be identified from their X-ray diffraction patterns. The lamellar phase shows a series of reflections corresponding to one-dimensional periodicity. From the X-ray data it is possible to determine the thickness of the liquid bilayer, the cross-section per polar headgroup and the water layer thickness. In the same way the diameter of the cylinder in the  $H_I$  and  $H_{II}$  phases can be determined as well as the cross-sectional area per polar headgroup and the distance between adjacent cylinders. These procedures are described in the fundamental work by Luzzati *et al* <sup>[126]</sup>.

### 1.7.3. Cubic Phase

The viscous isotropic cubic phase, which is periodic in three dimensions, exists mainly in monoglyceride-water systems at chain lengths above  $C_{14}$  (figure 1.11). It was originally thought to consist of spherical water aggregates arranged with cubic symmetry in a lipid matrix with the polar groups surrounding the water aggregates and the hydrocarbon chains filling the gaps between the water spheres <sup>[126]</sup>. Studies of the cubic monoglyceride-water phase indicated that the structure was closely related to that of the lamellar phase <sup>[127]</sup>. Furthermore, it was concluded from NMR diffusion measurement that the local environment of the lipid molecules was similar to the lamellar phase and different from phases with molecules arranged in rod system. It was also concluded that the structure was both lipid-and water-continuous, and was proposed to consist of lipid bilayer units with hexagonal shape connected into a

network. The hexagonal units would be expected to be curved in order to avoid sharp edges (figure 1.10d, far left from bottom). Later it was realized by Larsson *et al* <sup>[128]</sup>. That such a structure is identical to the primitive cubic alternative of an infinite periodic minimal surface (IPMS). The structure is shown in figure 1.10, to the right. The methyl endgroup gap of the bilayer is identical to the IPMS, and the infinite lipid bilayer thus formed separates two identical water channel systems. A new type of cubic structure has recently been reported by Luzzati *et al* <sup>[129]</sup>; it consists of two types of reversed micelles embedded in a continuous hydrocarbon-chain matrix. This phase is formed in many systems of water-swelling lipids mixed with lipids that do not form aqueous phases.

#### 1.7.4. Lipid-Water Phase Diagrams

##### 1.7.4.1. Binary-Phase Diagrams

Generally, phase equilibrium in binary lipid-water systems are illustrated by composition-temperature phase diagrams. There are two types of lipid-water phase diagrams. The first type is obtained from polar lipids whereby, the solubility in water is quite small; monolaurin for example has a solubility of about  $10^{-5}$ M. Figure 1.11 illustrates the principles of phase equilibrium in this type of lipid-water system. A homologous series of monoglycerides is used here as an example. Phase diagrams of pure 1-monoglycerides-water systems were first published by Lutten <sup>[131]</sup>, who described the phase behavior of monoglycerides with chain lengths from  $C_{12}$  to  $C_{22}$ . Larsson <sup>[132]</sup> described detailed phase diagrams for short-chain length monoglycerides  $C_6$ - $C_{10}$ . As shown in figure 1.11, at chain lengths  $C_8$ - $C_{12}$ , only the lamellar  $L_\alpha$  is formed. When the chain length is increased, there is also a cubic region with the two phases discussed in a previous section. Finally, at chain length above  $C_8$ , a phase termed  $L_2$  is formed when the temperature is increased.  $L_1$  is used for a normal micellar solution, whereas water aggregates in a hydrocarbon chain medium is termed  $L_2$  <sup>[133]</sup>. The second type of binary system is obtained when the lipid is soluble as micelles in water. Examples of such lipids are fatty acid salts and lysolecithin. An aqueous soap system is depicted in figure 1.12. When the lipid concentration in the micellar solution is increased, the spherical micelles are transformed into rod-shaped



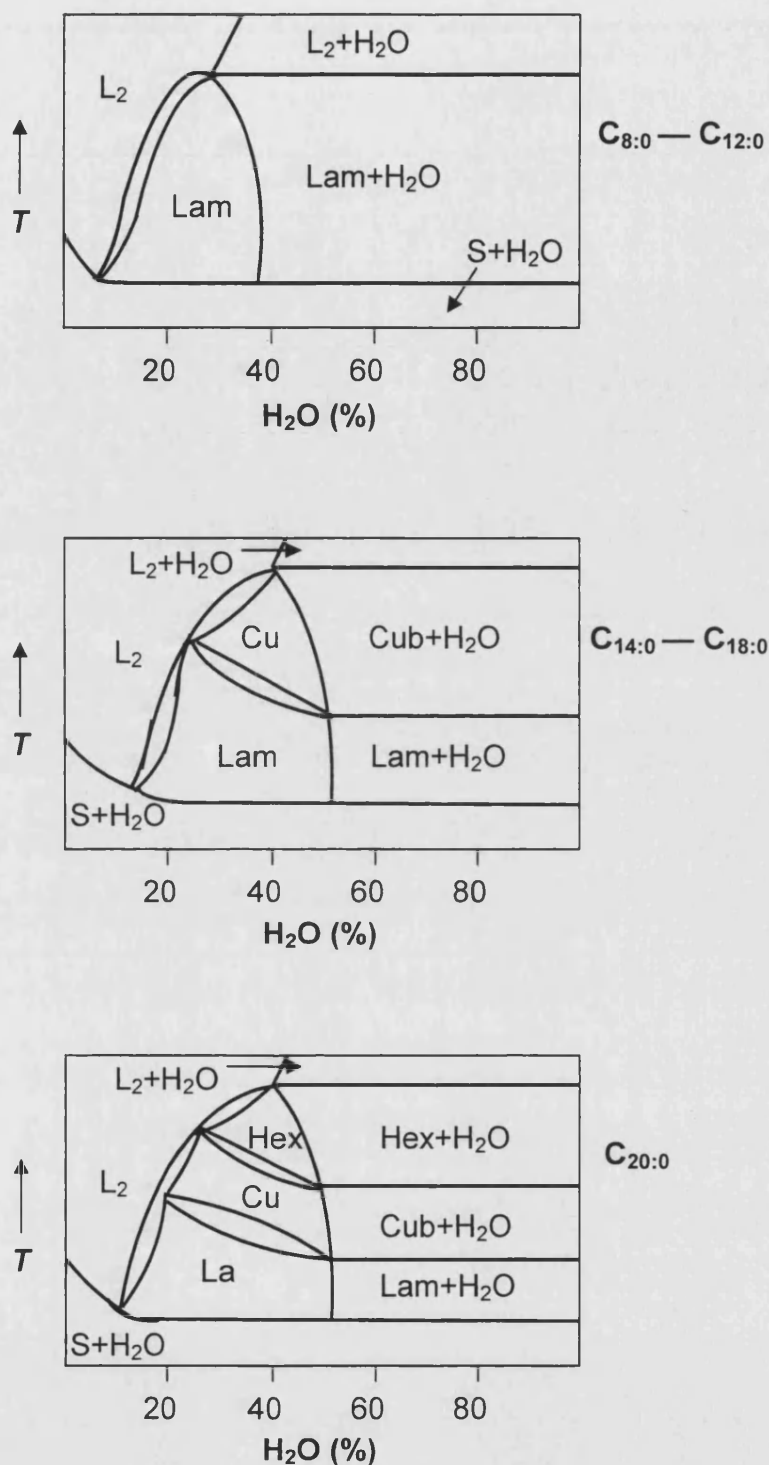
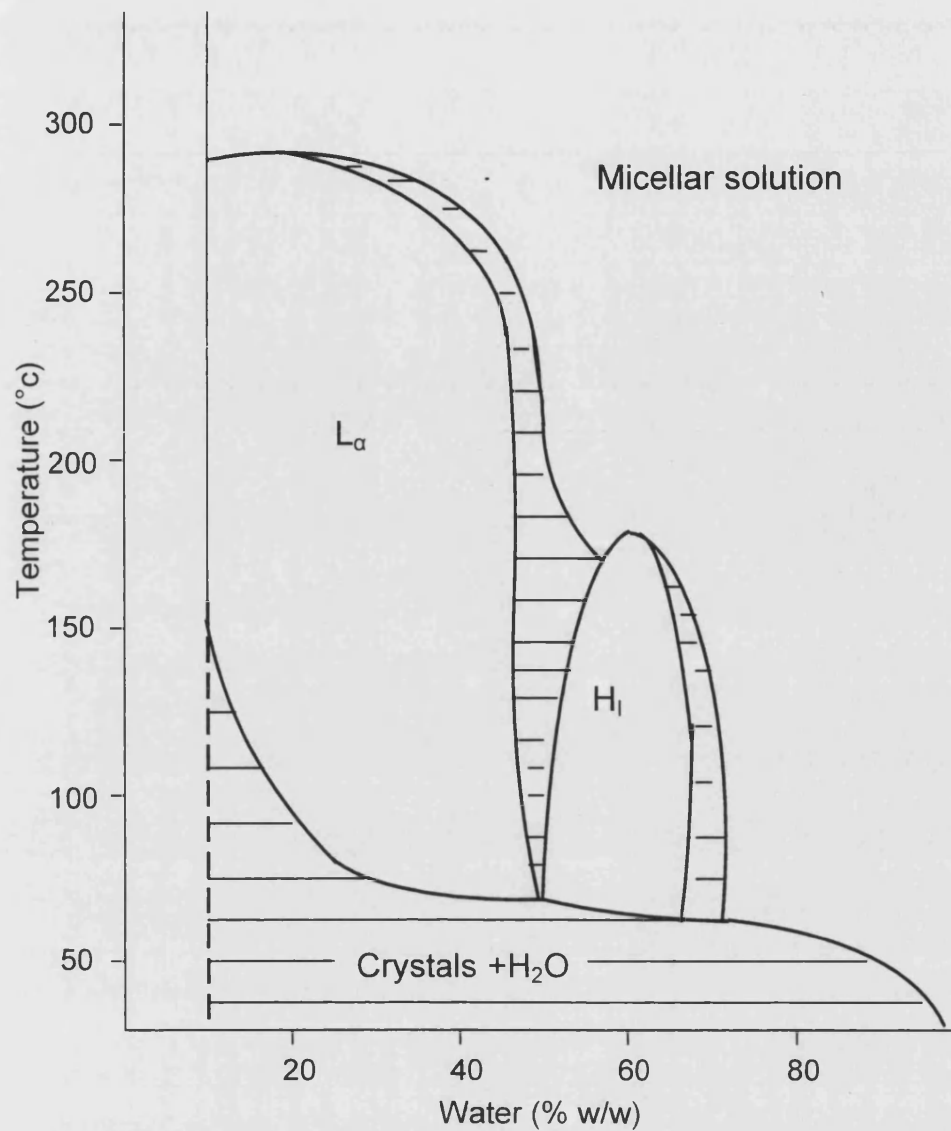


Figure 1.11

Main features of monoglyceride-water phase diagrams at different hydrocarbon chain lengths ( $\text{Lam}$ ,  $\text{Cu}$ , and  $\text{Hex}$  corresponds to regions of lamellar, cubic and hexagonal  $\text{H}_{\text{II}}$  phases, respectively). Below the hydrocarbon-chain melting temperature, there is solid ( $\text{S}$ ) material in equilibrium with water. Small shaded areas represent two phase regions (adapted from Ref. 130).



**Figure 1.12**

Phase diagram of sodium myristate-water. The phases are shown only to the right of the broken line at water concentrations >15% w/w (adapted from Ref. 130)..

micelles. At still higher lipid concentrations the lipid cylinders are hexagonally arranged and the liquid-crystalline phase is formed. The lamellar liquid-crystalline phase  $L_\alpha$  is usually formed in the region between  $H_1$  and the anhydrous lipid. Reviews of the association behavior of amphiphiles of this type have been published <sup>[134, 135]</sup>.

#### 1.7.4.2. Ternary-Phase Diagrams

A ternary system consists of a non-polar lipid such as triglycerides, water and an emulsifying agent. An example of such a ternary lipid system <sup>[136]</sup> is shown in figure 1.13. The triglyceride component, as well as the monoglyceride component, contains numerous compounds with different chains and both behave as one component with respect to the phase rule. The transition  $L_\alpha \rightarrow \text{Cubic} \rightarrow H_{II} \rightarrow L_2$ , obtained by heating the binary monoglycerides system (figure 1.11), is also obtained by solubilization of triglyceride molecules into the bilayer according to this phase diagram (figure 1.13). Increase in temperature or water content results in increased molecular disorder, and this disorder corresponds to an increased wedge shape of the average lipid molecule, as the disorder within the lipid molecule builds up towards the end of the hydrocarbon tail. The slope of the transition line  $L_\alpha$  to cubic and of the transition cubic to  $H_{II}$  in figure 1.11 clearly illustrates these effects. A quantitative theory relating the wedge shape of the amphiphile molecule to the phase that is formed with water has been introduced by Israelachvili *et al.* <sup>[137]</sup>.

#### 1.7.5 The Use of Liquid Crystal Vehicles in Pharmacology

Liquid crystal vehicles offer the possibility of dissolving water soluble, non-water soluble and amphiphilic compounds. Substances which are soluble in any of the phases and which go through extensive degradation in an acid environment increase their stability when incorporated in a liquid crystal matrix. The matrix structure does not permit gastric juice to enter the vehicle, which thus prevents degradation <sup>[138]</sup>. Extended release of larger water-soluble substances and non-polar substances is easily managed with liquid crystal-based vehicles. The actives are trapped in the liquid crystal formulation. When the liquid crystal vehicle comes into contact with the gastrointestinal fluids, the drug from the surface immediately starts to dissolve and

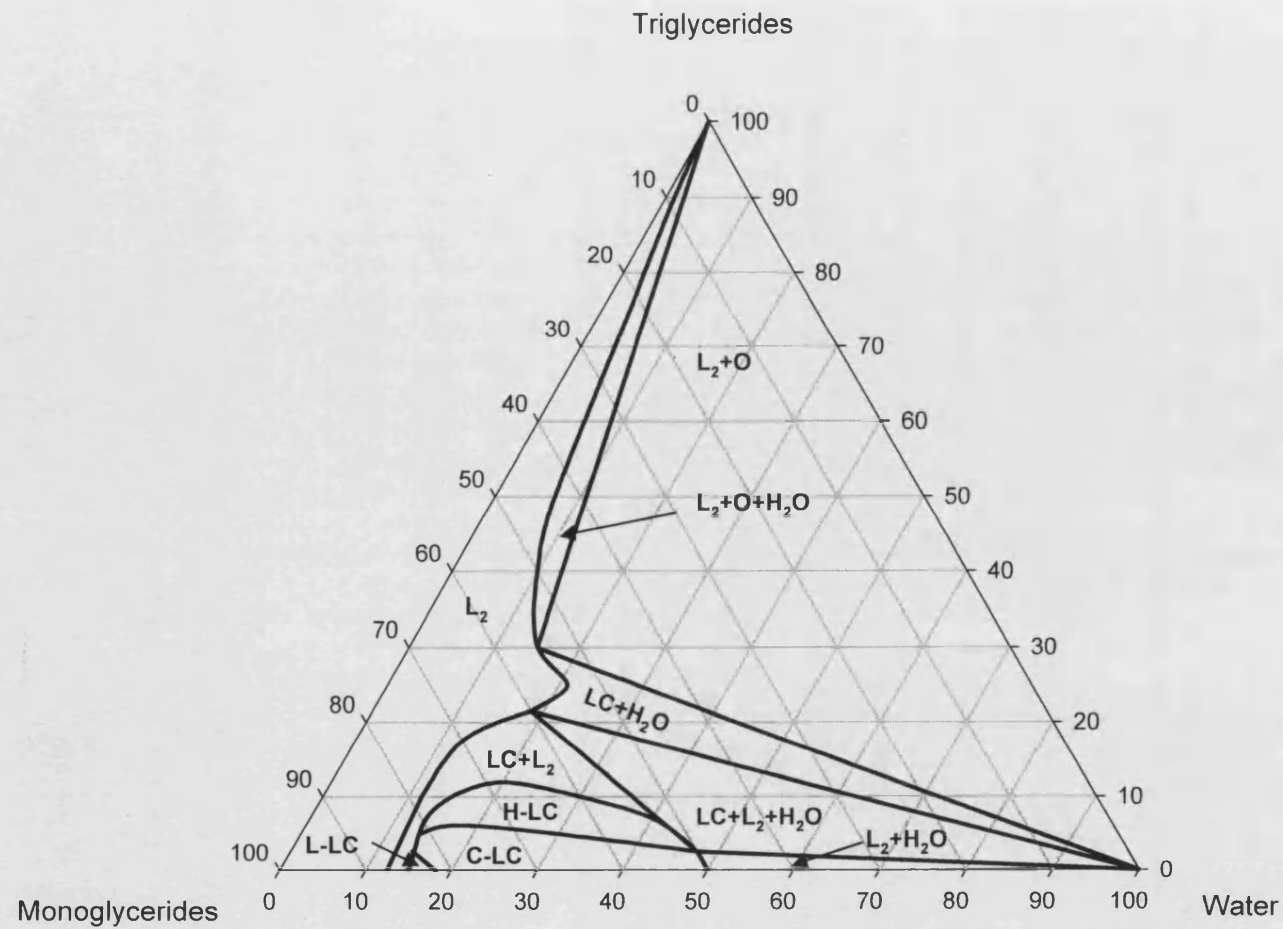


Figure 1.13: Phase equilibrium of the ternary system soybean oil-water-monoglycerides of sunflower oil. The liquid crystalline (LC) phases are Lamellar (L-LC), cubic (C-LC) and hexagonal (H-LC), axes are in percentages (adopted from Ref. 136).

reaches the surroundings liquid. As the drug slowly clears and dissolution progresses, new surface zones appear-releasing the next layer of drug, and so on. The dissolution does not occur instantly at body temperature it can take hours. Optimization of release rate is therefore needed. In the dilution process in the gastrointestinal fluids, liquid crystals are dissolved and transformed to an emulsion or a microemulsion / micellar system depending on the type of surfactant used. The transformation increases the solubility and effective area of the poorly soluble substances in the gastrointestinal lumen, which in turn increases the bioavailability.

An excellent review has recently been published by Drummond *et al.* <sup>[139]</sup> outlining the application of surfactant self-assembly objects based on hexagonal, cubic, 'intermediate' and L<sub>3</sub> ('sponge') phase structures as novel drug delivery vehicles.

Norling *et al.* <sup>[140]</sup> have described the development of a stable, sustained release formulation of an antimicrobial agent, metronidazole, to treat periodontol disease. The product is based on a mixture of monoglycerides and triglycerides and formulated as water-free suspension that transforms to the reversed hexagonal phase in the periodontol pocket. The reversed hexagonal phase has been observed to form upon the complexation of cationic and neutral lipids with DNA in aqueous solution <sup>[141]</sup>. This work by Kotover *et al.* <sup>[141]</sup> suggests that there is a possibility that non-lamellar lyotropic liquid crystalline phases may be utilized in the future as a non-viral method of gene delivery.

Glyceryl monooleate (monoolein or GMO), a Food and Drug Administration-approved food additive, is a mixture of the glycerides of oleic acid and other fatty acids, consisting mainly of the monooleate, and has the ability to form different types of lyotropic liquid crystals in the presence of water <sup>[142]</sup>. The use of liquid crystalline phases of GMO as drug delivery systems has been widely investigated by many co-workers <sup>[142-145]</sup>. The unique properties of cubic liquid crystalline phases formed from GMO systems have been utilized for the preparation of controlled release systems; and in topical and mucosal drug delivery systems due to their adhesive properties. A wide variety of drugs with different physicochemical properties have been incorporated in GMO <sup>[139]</sup>. A recent study by Sallam *et al.* <sup>[146]</sup> has investigated the properties of the cubic liquid crystalline phases of GMO to formulate an oral drug delivery system for furosemide.

Liquid crystalline mesophases with rhombohedral, monoclinic and tetragonal symmetry, also known as 'intermediate' phases, have been identified in some surfactant-water system <sup>[147]</sup>, e.g. sodium dodecyl sulfate (SDS)-water system. Engström *et al* <sup>[148]</sup> have also reported the formation of 'sponge' phases in solvent-GMO-water systems. The L<sub>3</sub> or 'sponge' phase is considered to be a disordered version of the cubic phase <sup>[149, 150]</sup>. However, these exotic mesophases, termed 'intermediate' and 'sponge' phases, have not yet been investigated as drug delivery systems.

## **1.8. Bioavailability Control of Poorly Absorbed Drugs.**

The enhancement of oral bioavailability of poorly water-soluble drugs remains one of the most challenging aspects of drug development. Salt formations, particle size reduction, solubilization, and solid dispersion have been used to increase dissolution rate and thereby oral absorption and bioavailability of such drugs <sup>[151]</sup>. The salt formation is not feasible for neutral compounds and the synthesis of appropriate salt forms of drugs that are weakly acidic or weakly basic may often not be practical. Yet when salts can be prepared, re-conversion of salts into aggregates of their respective acid or base might occur thus an increased dissolution rate can not necessarily be achieved. Particle size reduction is commonly used to increase dissolution rate, however there is a practical limit to how much size reduction can be achieved by controlled crystallization, grinding, etc. The use of very fine powders in a dosage form may also raise problems because of handling difficulties and poor wettability. Administration of a hydrophobic drug dissolved in a lipidic solvent or using solid dispersion method is thought to improve bioavailability and hence a more consistent profile of drug absorption with time is observed. These last two methods will be discussed in more details in following subsections.

### **1.8.1. Absorption of Drugs from Solution**

The dissociation constant and lipid solubility of drug, as well as the pH at the absorption site, dictate the absorption characteristics of a drug from solution. The interrelationship among these parameters is known as the pH-partition theory of drug

absorption. This theory is based on the assumption that the gastrointestinal tract is a simple lipid barrier to the transport of drugs and chemicals. Accordingly, the nonionized form of an acid or basic drug, if sufficiently lipid-soluble, is absorbed but the ionized form is not. The larger the fraction of drug in the nonionized form at a specific absorption site, the faster is the absorption. Acid and neutral drugs may be absorbed from the stomach but basic drugs are not. The rate of absorption is related to the oil-water partition coefficient of a drug; the more lipophilic the compound, the faster is its absorption.

#### 1.8.1.1. Drug $pK_a$ and Gastrointestinal pH

The fraction of drug in solution that exists in the nonionized form is a function of both the dissociation constant of the drug and the pH of the solution. The dissociation constant is often expressed for both acids and bases as a  $pK_a$ . The  $pK_a$  values of several drugs and the relative acid or base strengths of these compounds are shown in figure 1.14. The relationship between pH and  $pK_a$  and the extent of ionization is given by the Henderson-Hasselbalch equation:

for an acid

$$pK_a - pH = \log (f_u/f_i)$$

and for a base

$$pK_a - pH = \log (f_i/f_u)$$

where  $f_u$  and  $f_i$  are fractions of the drug present in the un-ionized and ionized forms, respectively. Most acid drugs are predominantly un-ionized at the low pH of gastric fluids (see figure 1.15) and may be absorbed from the stomach as well as from the intestines. The pH range found in the GIT from the stomach to the colon is about 1 to 8. Very weak acids ( $pK_a > 8$ ) such as, phenytoin, theophylline, or glutethimide are essentially un-ionized throughout the GIT thus, they are very much equivalent in the gut to non-electrolytes. The ionization of weak acids with  $pK_a$  values ranging from about 2.5 to 7.5 is sensitive to changes in pH. More than 99% of the weak acid aspirin ( $pK_a = 3.5$ ) exist as un-ionized drug in gastric fluids at pH 1. On the other hand, only about 0.1% of aspirin is un-ionized at pH 6.5 in the fluids of the small intestine. Despite this apparently unfavorable ratio of non-ionized to ionized drug, aspirin and most weak acids are well absorbed in the small intestine. A large surface area and a relative long residence time in the small intestine are contributing factors.

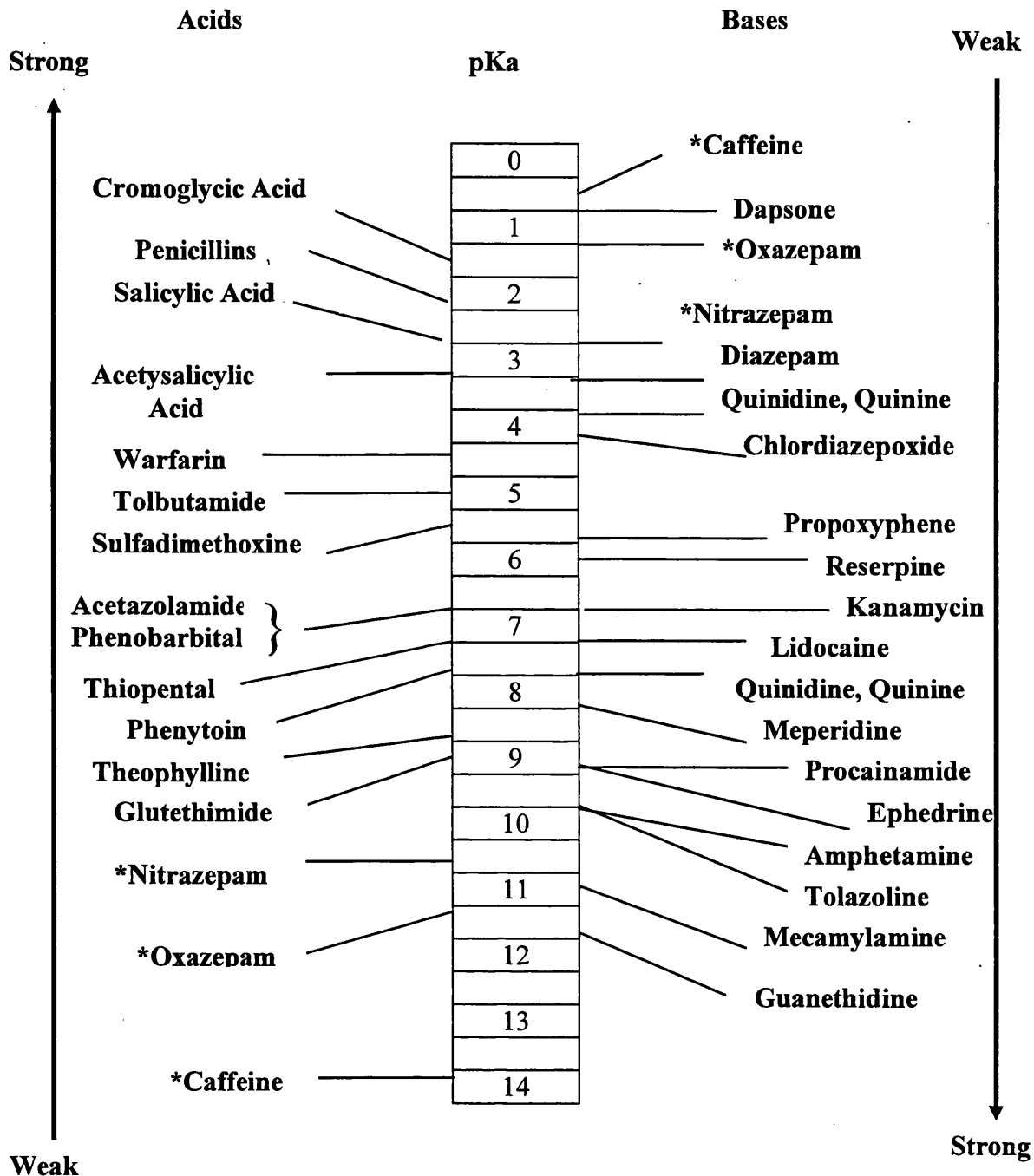


Figure 1.14:

The pKa values of certain acidic and basic drugs. Those drugs denoted with an asterisk (\*) are amphoteric (adapted from Ref. 152).



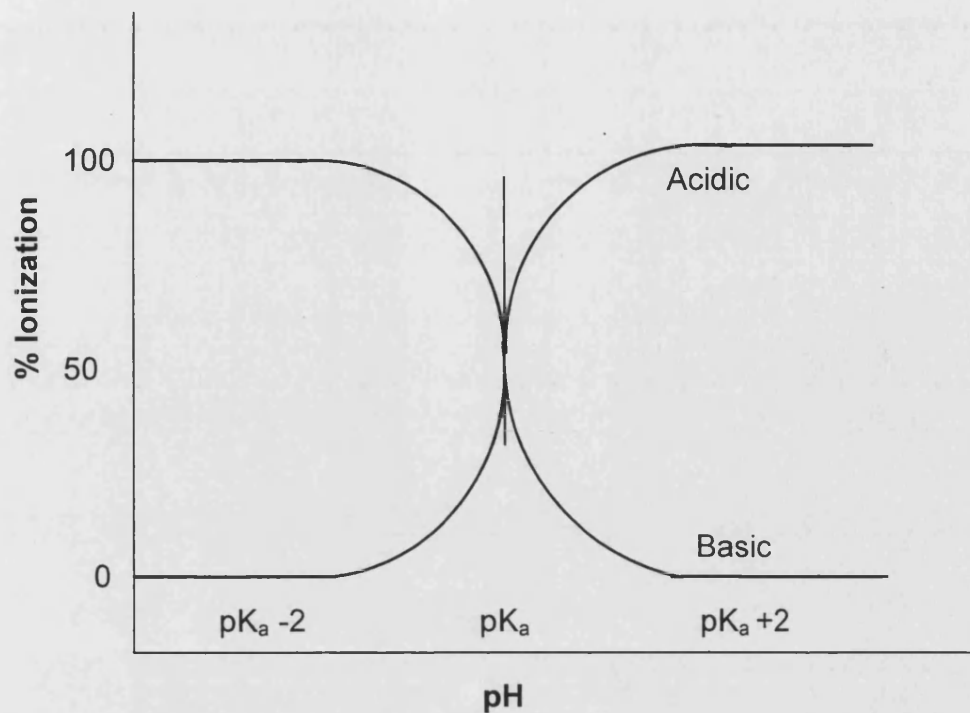


Figure 1.15: Ionization profiles of weak acidic and basic compounds against increasing pH.

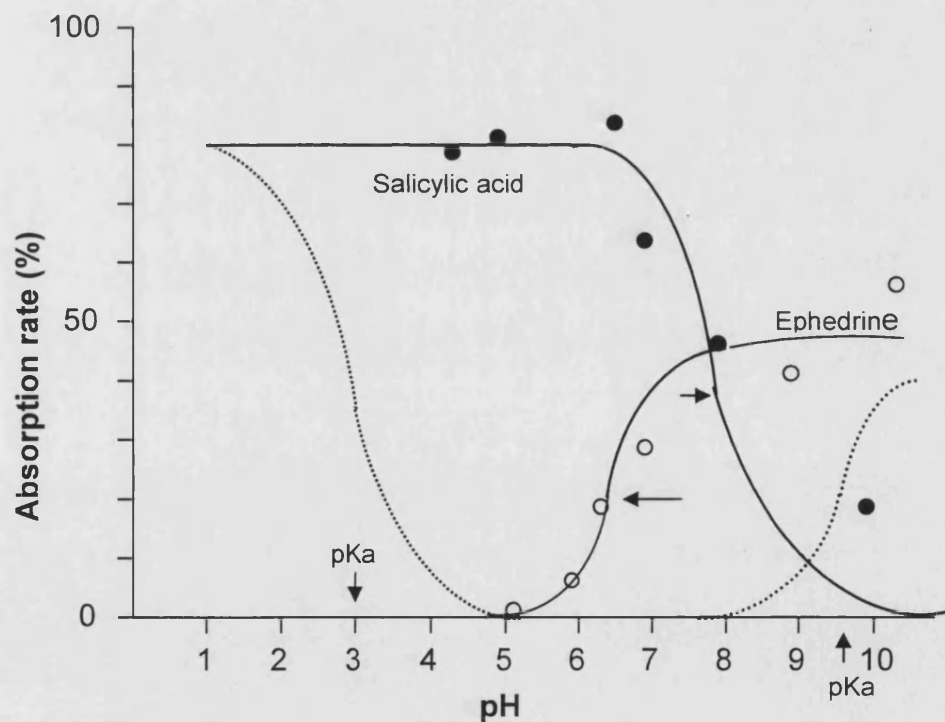


Figure 1.16: Relationship between absorption rate of salicylic acid ( $pK_a = 3$ ) and ephedrine ( $pK_a = 9.5$ ) against bulk phase pH in the rat small intestine in vivo. Dashed lines indicate curves predicted by the pH-partition theory in the absence of an unstirred layer (adapted from Ref. 153).

These factors minimize the need for a large fraction of the drug in an un-ionized form in the small intestine. Strong acids (such as cromolyn  $pK_a$  2) are ionized throughout the GIT and are poorly absorbed.

Most weak bases are poorly absorbed, if at all, in the stomach since they are largely ionized at low pH. Codeine, a weak base with a  $pK_a$  of about 8 will have only 1 of every million molecules in the non-ionized form in gastric fluid as it is mainly ionized at pH 1. The pH range of the intestines from the duodenum to the colon is about 5 to 8. Weakly basic drugs ( $pK_a < 5$ ), such as dapson, diazepam, or chlorodiazepoxide, are essentially un-ionized throughout the intestines, this makes them more equivalent to non-electrolytes in intestines. Stronger bases, such as mecamlamine or guanethidine are ionized throughout the GIT and tend to be poorly absorbed.

The pH-partition theory provides a basic frame work for understanding drug absorption; however it is an oversimplification of a more complex process. The theory indicates that the relationship between pH and permeation or absorption rate is described by an S-shaped curve corresponding to the dissociation curve of the drug. For a simple acid or base, the inflection point of the pH-absorption curve should occur at a pH equal to the  $pK_a$  of the drug. This is rarely observed experimentally. In general, pH-absorption curves are less steep than expected and are shifted to higher pH values for acids and to lower values for bases. The pH-absorption curves for salicylic acid (a weak acid with a  $pK_a$  of about 3) and for ephedrine (a weak base with a  $pK_a$  of about 9.5) have inflection points at about pH 8 and pH 6.5 <sup>[153]</sup> respectively, see figure 1.16. Theory predicts little absorption of salicylic acid at pH 8 because at this pH the drug would be almost completely ionized. As shown in figure 1.16, the absorption rate of salicylate across the small intestine at pH 8 is about 50% of the maximum absorption rate. Several factors may contribute to the deviations from the pH-partition theory; the absorption of the ionized form of the drug, the presence of an unstirred diffusion layer adjacent to the cell membrane, and a difference between luminal pH and the pH at the surface of the cell membrane.

#### **1.8.1.2. Absorption of Hydrophobic Drugs from GI Tract**

The rate of absorption of non-electrolyte from the crystalline state depends on drug dissolution rate and the intrinsic rate of absorption across the epithelia of the intestine.

As depicted in figure 1.17, it is clear that dissolution for hydrophobic drugs is a rate limiting step. In the case of crystalline non-electrolyte compounds (Figure 1.17a), dissolution is too slow due to low intrinsic water solubility. However, many drugs are weak electrolytes and often absorbed to an adequate extent because their  $pK_a(s)$  allow them to exist predominantly as ionized species in the lumen of the gut. In such cases the drug is absorbed as the unionized species but the ionized species represents a reservoir of drug from which the unionized species is immediately available. Therefore, the slow dissolution of the unionized species is avoided (figure 1.17b). Weak bases, for this discussion with  $pK_a$  less than 7, will not be highly soluble in the small intestine, and indeed may dissolve in the stomach, only to precipitate after gastric emptying delivers them to the small intestine. Thus when weak bases are administered in a solid dosage form, they will not benefit from a substantial reservoir of dissolved drug. Their absorption is likely to be dissolution rate-limited, analogous to biopharmaceutical properties of non-electrolytes. Therefore, when the drug is a non-electrolyte or an electrolyte with  $pK_a(s)$  which are unfavorable for dissolution in the gut, there are a number of formulation approaches one can take to improve absorption. One commonly used technique is to solubilise the drug in a water-miscible co-solvent system, such as polyethylene glycol. This may have sufficient solvent capacity for the drug in the unit dosage form. However, there is a tendency that the drug will crystallize on dilution of the co-solvent in the lumen of the gut. This has been elucidated in later chapters of this thesis. The effect of the formulation strategies on the absorption of non-electrolytes or electrolytes with unfavorable  $pK_a(s)$  from the GIT is presented in figure 1.18. The co-solvent approach could be convenient if the drug precipitates as a microfine suspension after dispersion finer than could be achieved by micronisation. A better strategy is to maintain solubility of the drug throughout its passage through the gut. As shown in figure 1.18, this can be achieved using different types of lipid systems (Type I, II and III), assuming that they provide sufficient solvent capacity to dissolve the drug in a small volume for administration as a unit dose. As a consequence, non-electrolytes, weak acids with  $pK_a > 8$  and weak bases with  $pK_a < 5$  may benefit from reformulation in lipids, which can provide a reservoir of drug dissolved in either lipid or micellar solution (figure 1.19). Some of these formulations will be expected to be digested during their passage through the GIT. Therefore, the mechanism by which the drug is maintained in

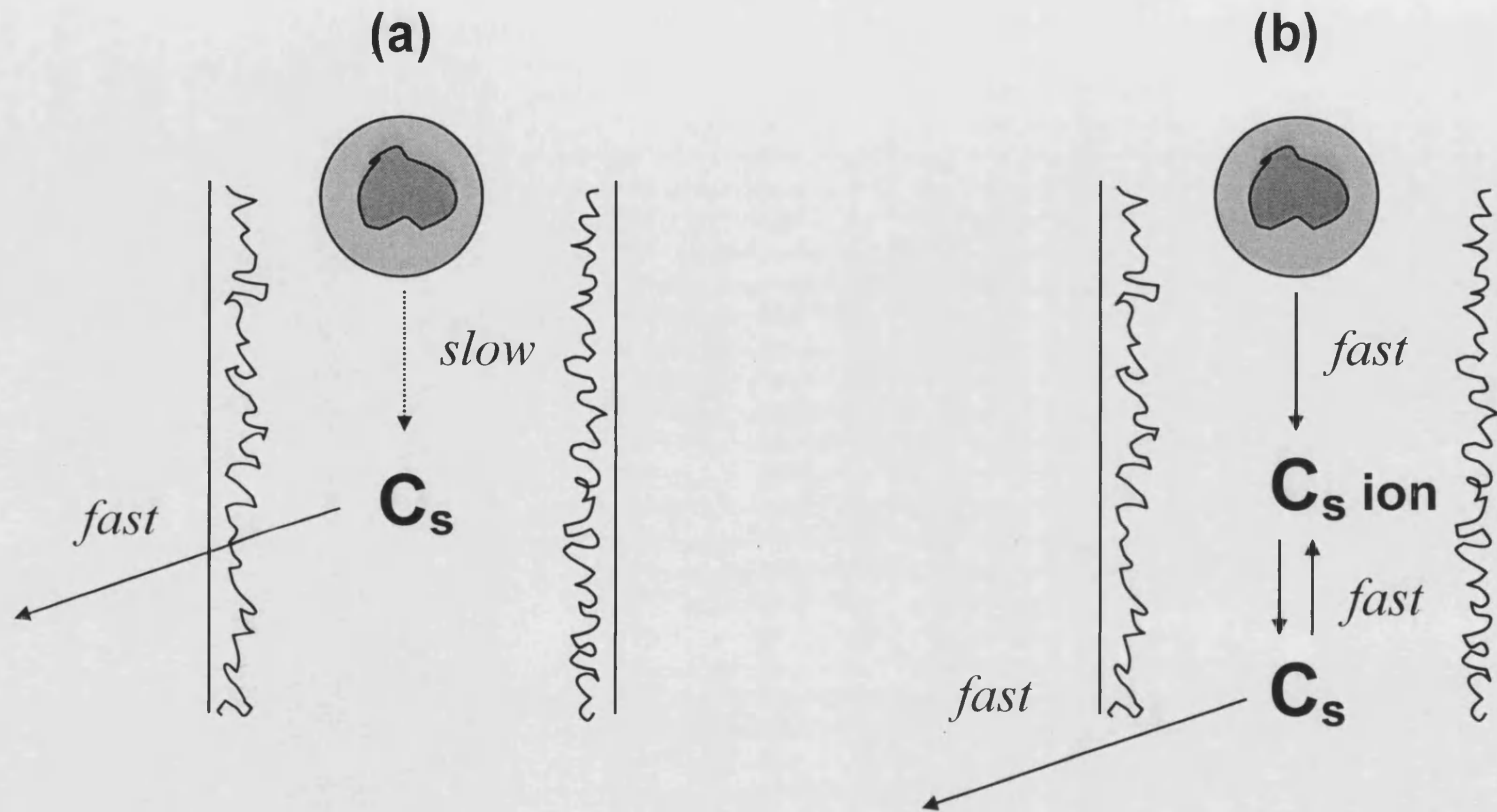


Figure 1.17

Gastro-intestinal absorption of hydrophobic crystalline drugs: (a) non-electrolytes and (b) weak electrolytes; if  $pK_a$  is appropriate ionized drug provides a reservoir of unionized drug (adapted from Ref. 104).

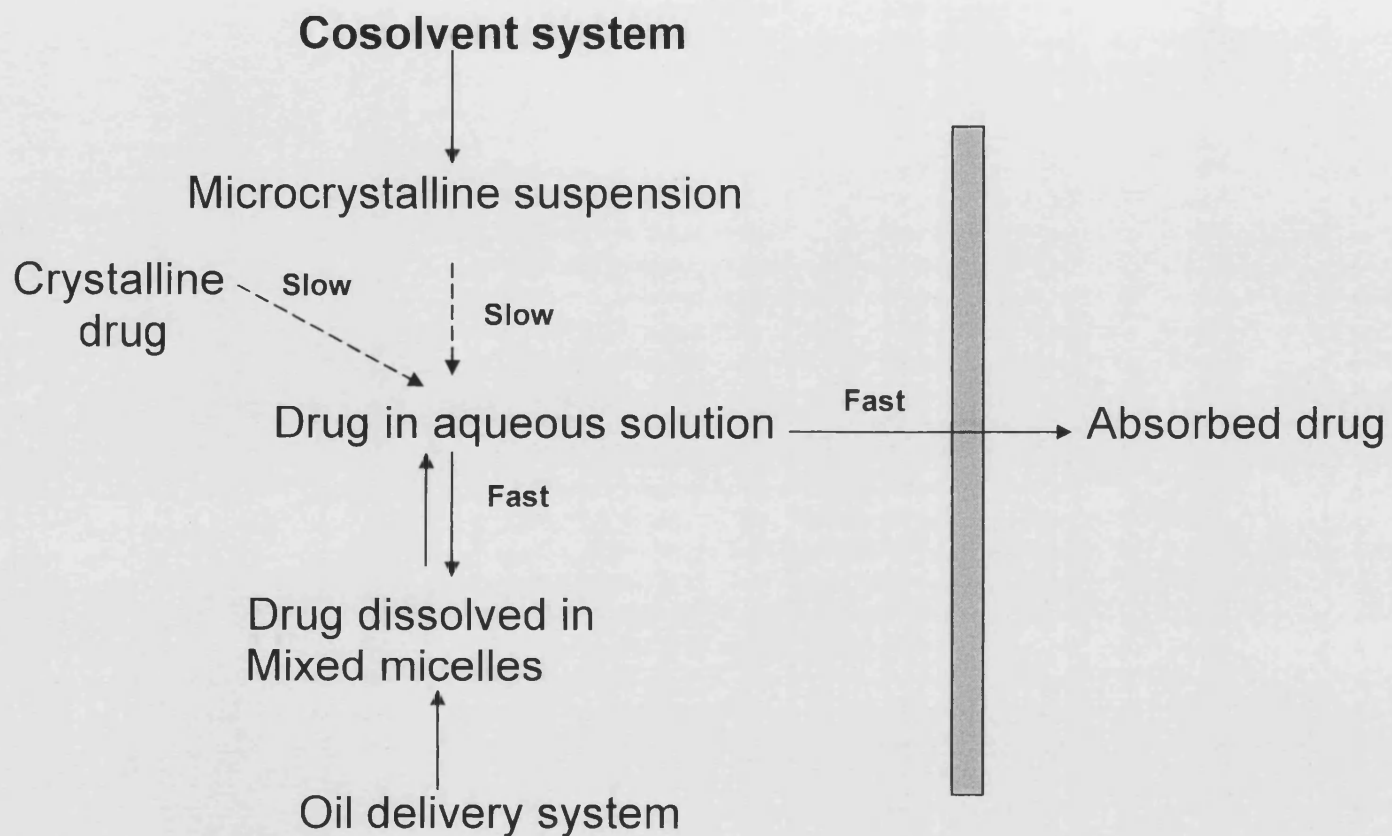


Figure 1.18

Schematic diagram of the influence of formulation strategies on the GI tract absorption of crystalline hydrophobic non-electrolytes or electrolytes with unfavourable pKa (s) (adapted from Ref. 154).

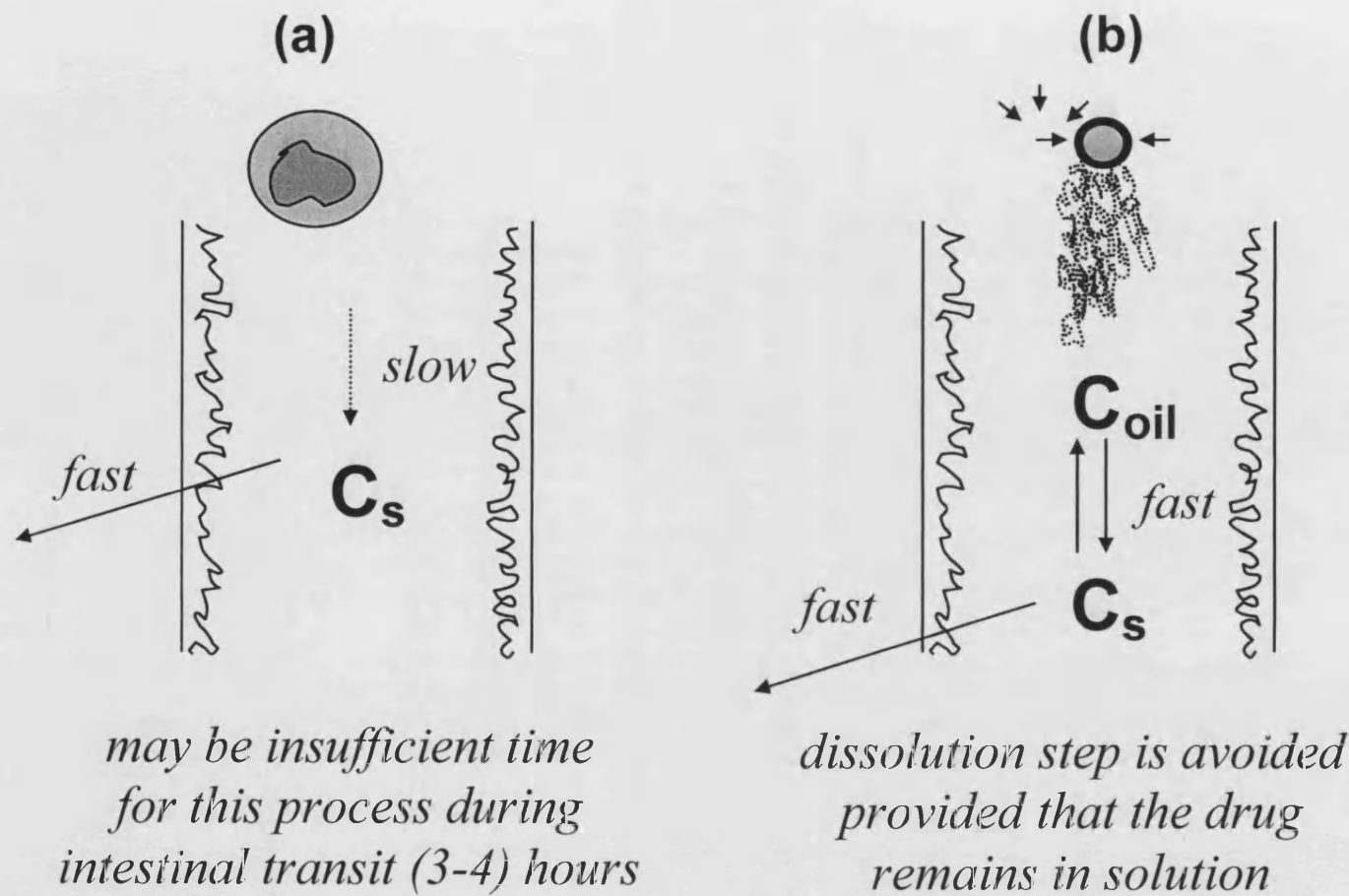


Figure 1.19

Gastro-intestinal absorption of hydrophobic drugs as (a) crystalline forms (b) oil systems. Self-emulsified drug delivery systems (SEDDS) can provide a reservoir of drugs in oil (adapted from Ref. 104).

solution will depend on the solubilization of drug by mixed micelles of bile and the products of lipolysis. This will be further discussed in following subsections.

#### **1.8.1.3. GI Tract Absorption of Drugs from Digestible Oil Formulations**

Following passage into the small intestine, the formulation will become exposed to local secretions of lipase and bile salts from the pancreas and gall-bladder respectively. These secretions will together facilitate lipolysis, wherein orally ingested triglycerides are rapidly digested in the upper small intestine, through the integrated action of pancreatic lipase, colipase and bile. Colipase binds to the surface of fat droplets in the presence of bile salts and provides an attachment site for lipase <sup>[155]</sup>. Lipase sequentially attacks the two outside ester bonds of the triglyceride molecule, producing first a molecule of diglyceride and fatty acid, and then a molecule of monoglyceride and fatty acid. The lipolysis products are removed from the oil-water interface and incorporated into mixed micelles by bile salts <sup>[156]</sup>. Thus, in effect, a direct pathway exists for the flow of solubilized drug from digesting oil droplets to the final product phase, mixed micelles, which provide a strong solubilizing environment for hydrophobic drugs. This provides the reservoir of drug from which partitioning will occur, allowing absorption of free drug from the lumen of the gut, see figure 1.20. The rate of lipolysis depends mainly on the size of emulsion particles. In order to maximize the rate of drug partitioning into aqueous intestinal fluids, and hence the absorption rate, the formulation should be highly dispersible.

#### **1.8.2. Bioavailability Enhancement Using Lipid Systems**

Most drugs are administered orally as solid dosage forms either as tablets or powder filled hard gelatin capsules. Before the drug can be absorbed it must pass through a number of stages presented in figure 1.21. Lipophilic drug lipid delivery systems can have biopharmaceutical advantages over conventional tablet or powder-filled hard gelatin capsules. The dissolution process from solid dosage forms often limits the rate of absorption of the drug; if this step is removed, improved bioavailability can result. This increase in control of absorption from the gastro-intestinal tract can therefore provide a more effective dosage. The use of a simple solution of drug in lipid is rarely possible due to the limited solubility of some hydrophobic drugs in lipids.

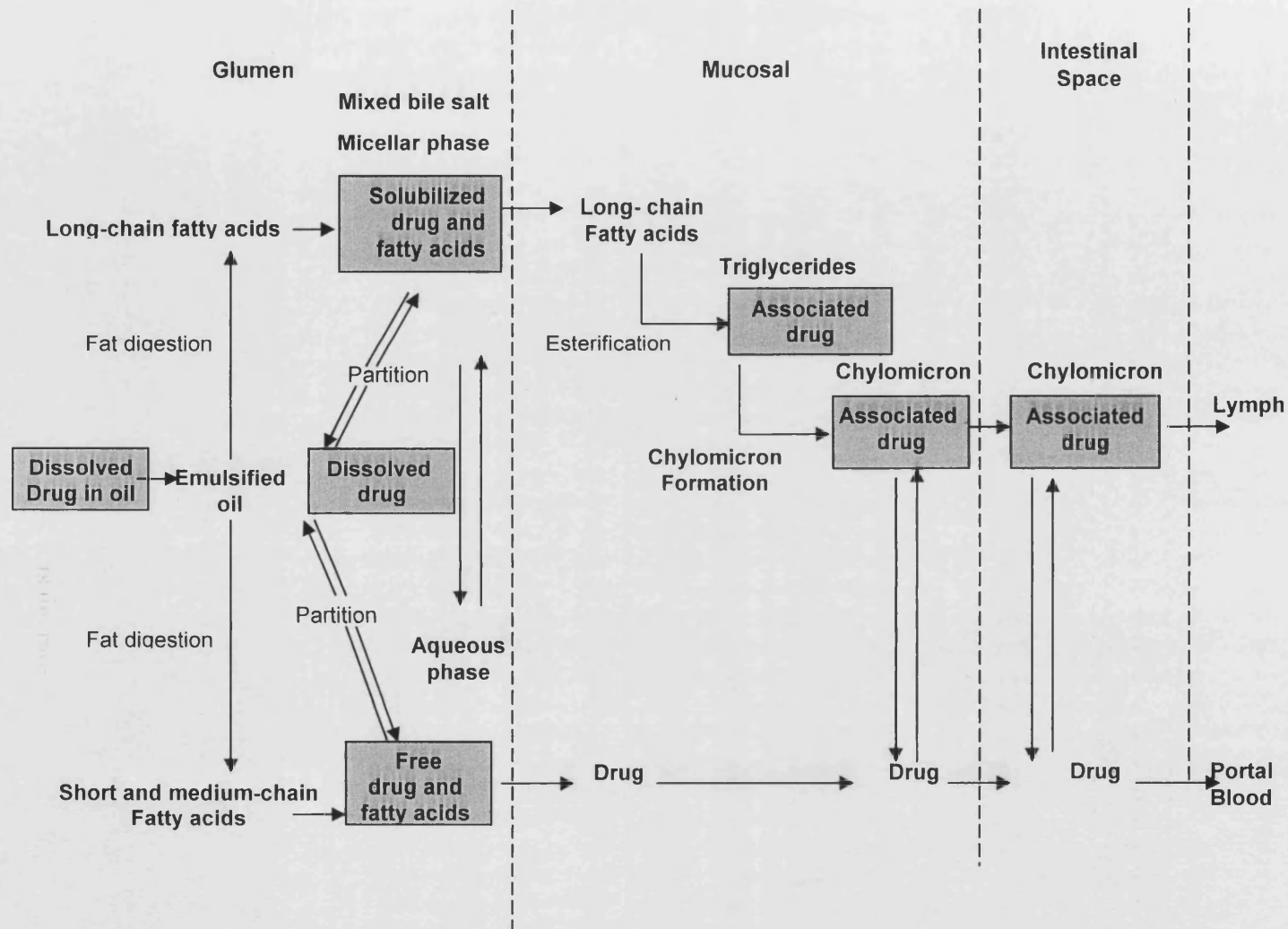


Figure 1.20: Pathways for the absorption of drugs into the lymphatic and venous system (adapted from Ref. 157).



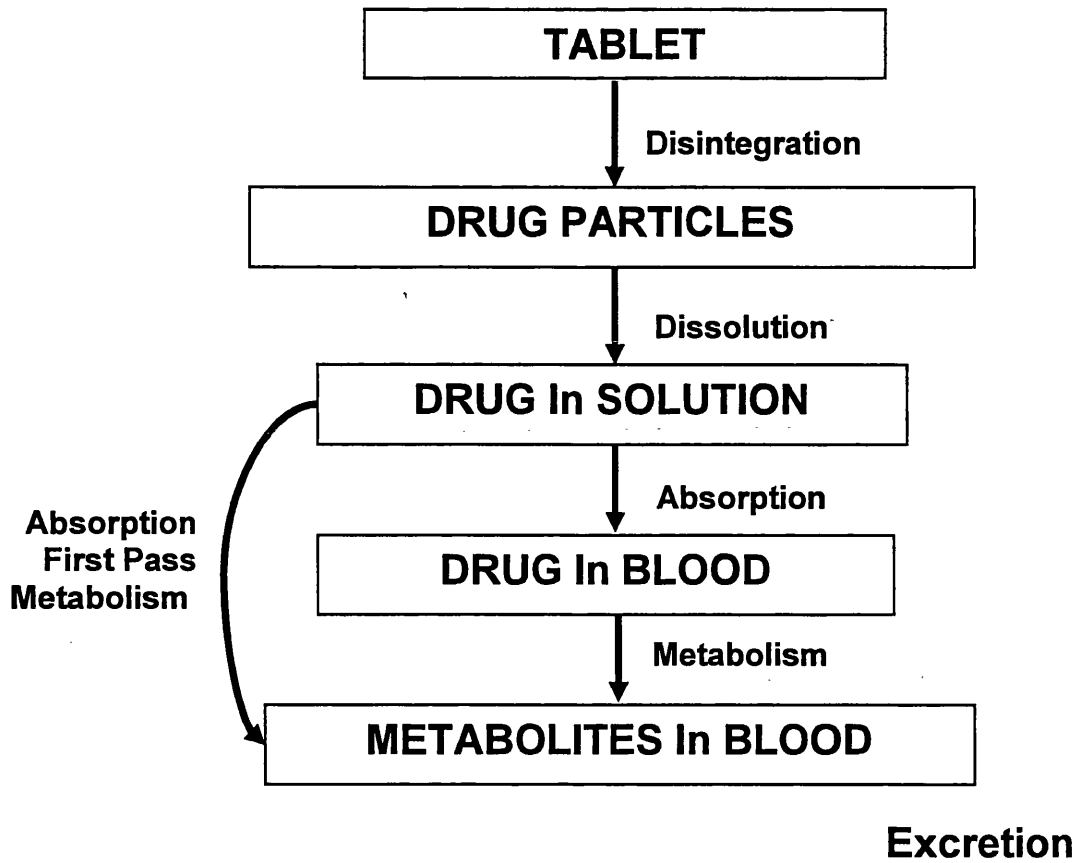


Figure 1.21

Disintegration, dissolution and absorption processes of a drug from a solid dosage form.

This generally leads to the design of more complex lipid-based formulations containing other components such as co-solvents. Administration of medicinal compounds as solutions in oil may still lead to erratic bioavailability as the degree of emulsification that occurs *in-vivo* can be very variable or may not occur. This depends on the state of the stomach contents (fed or fasting) as well as the level of enzymes, acid and bile salts present in the gastro-intestinal tract at the time of dosing. The alternative is to present the lipophilic drug in a surfactant-oil-cosolvent mixture (SEDDS or SMEDDS) which, on contact with water forms an emulsion or microemulsion with minimal energy input. Dispersion of the oil as small droplets results in much larger interfacial area between oil and water. Hence the rate of drug transfer by diffusion from the oily to the aqueous phase (gastro-intestinal tract fluid) is increased. On oral ingestion of such a dosage form, the gentle agitation provided by the gastro-intestinal tract would cause emulsification of the oily solution once the soft elastic gelatin capsule had been ruptured. More recently, lipid mixtures are being formulated in sealed hard gelatin capsule forms. It is potentially possible to control the resulting oil droplet size distribution after the dispersion of the formulation by altering the constituents and the composition of the self-emulsifying surfactant-oil mixture. The bioavailability of a given drug candidate will be governed by the resultant available surface area for diffusion of the drug from the oil into the aqueous phase, which is defined by the emulsion droplet size distribution, and the partition coefficient of the compound between aqueous and oily phases <sup>[158]</sup>. If absorption was not limited by the gut membrane, then the ratio of absorption of a drug would be greater from a microemulsion. Secondary factors which can modify the rate and the route of absorption of a drug from an oily vehicle relate to the digestibility of the oil and the lipophilicity of the drug.

As early stimulus for the use of lipid vehicles for drug formulation were the findings of improved bioavailability when poorly-water soluble drugs were co-administered with food. This prompted investigation of simple lipid solutions and suspensions of powdered drug. Well known examples include corn oil suspensions of griseofulvin <sup>[159]</sup> and phenytoin <sup>[160]</sup>. These simple formulations improved oral bioavailability compared to administration of an aqueous suspension. Abrams *et al.* studied the oral proportionality of a lipophilic steroid derivative when administered to rats as either solution or suspension formulations using sesame oil <sup>[161]</sup>. The bioavailability from a

sesame oil solution was three-fold higher than when the same dose was administered as an aqueous suspension of solid drug.

Bloedow and Hayton <sup>[162]</sup> have suggested that polar digestible lipids increases the bioavailability of lipophilic poorly water soluble drugs, while non-digestible lipids such as mineral oil (liquid paraffin) and sucrose polyesters have no effect on bioavailability and may however decrease the absorption rate. Non-digestible lipids essentially remain unabsorbed in the intestinal lumen, and can actually limit or reduce drug absorption by retaining a proportion of the co-administered drug <sup>[163]</sup>. Myers and Stella <sup>[108]</sup> investigated the effects of non-digestible and digestible lipid vehicles on the oral bioavailability of penclofedine, a highly lipophilic cytotoxic agent. Highest bioavailability was from trioctanion due to rapid digestion of MCT, lower bioavailability from the LCT due to slower and incomplete digestion, and still lower bioavailability from the non-digestible mineral oil. However, bioavailability from short chain triglyceride (SCT) tributyrin vehicle was very low, despite the expected rapid digestion of the SCT lipid. It was suggested that digestion of tributyrin was followed by rapid dissipation of tributyrin and its water soluble digestion products. This would predispose the associated proportion of administered drug to precipitation as crystalline drug with the same inherent dissolution limitations as the aqueous suspension formulation.

Oil digestibility can alter the pathway by which a compound is removed from the gastrointestinal mucosa. Fatty acids of carbon chainlengths greater than 14 are considered to be assembled into chylomicrons and transported by the lymphatic system. Carbon chainlengths between 8 and 12 diffuse passively into the systemic circulation via the portal vein <sup>[164]</sup>. Major routes for absorption of a lipophilic drug from an oil based formulations are summarized in figure 1.20. Palin and Wilson examined <sup>[109]</sup> the effect of different lipids on the absorption of probucol. The drug was administered as a LCT solution (arachis oil), a MCT solution (miglyol) or a liquid paraffin solution. Plasma concentrations were markedly higher after administration as an arachis oil solution compared with a miglyol solution, and were negligible from the liquid paraffin vehicle. It was concluded that digestion of the vehicle facilitated absorption, the subsequent incorporation of the long chain fatty acids into the lipoprotein-dependent (chylomicron) lymphatic transport path, contributed the superior bioavailability when probucol was co-administered with arachis oil.

Emulsification of the lipid vehicle has the obvious advantage of increasing the surface area for release of drug from the vehicle. Furthermore, emulsification will increase the available surface area for binding of the lipase/colipase complex thereby facilitating the kinetics of lipid digestion, while the presence of interfacially active components could also affect lipase binding and associated processes. A new lipid-based formulation of cyclosporin (Sandimmune Neoral<sup>®</sup> which contains drug, hydrophilic surfactant, co-solvent and lipids) produces a finely dispersed microemulsion when diluted in an aqueous phase. This microemulsion pre concentrate formulation was shown to increase the oral bioavailability of cyclosporin by an approximately 2-fold factor, compared with the previous commercial capsule formulation (Sandimmune<sup>®</sup> which contains drug, corn oil, ethanol and polyglycolised long chain triglyceride) and which produces a crude oil-in-water emulsion when added to water <sup>[165]</sup>.

### 1.8.3. Bioavailability Enhancement Using Solid Dispersions

The use of solid dispersions of drugs in water-soluble carriers to increase the dissolution rate and hence bioavailability of poorly soluble drugs has been studied extensively <sup>[166, 167]</sup> since the early work of Sekiguchi and Obi <sup>[168]</sup> in 1961. This method, which was later termed solid dispersion <sup>[166]</sup>, involved the formation of eutectic mixtures of drugs with water-soluble carriers by the melting of their physical mixtures. Sekiguchi and Obi <sup>[168]</sup> suggested that the drug was present in a eutectic mixture in a microcrystalline state. Later, Goldberg *et al.* <sup>[169, 170]</sup> demonstrated that all the drug in a solid dispersion might not necessarily be present in a microcrystalline state; a certain fraction of the drug might be molecularly dispersed in the matrix, thereby forming a solid solution. In either case, once the solid dispersion is exposed to aqueous media and the carrier dissolves, the drug is released as very fine colloidal particles. Because of greatly enhanced surface area obtained in this way, the dissolution rate and the bioavailability of poorly water-soluble drugs are expected to be high. Sheen *et al.* <sup>[171]</sup> enhanced the bioavailability of a poorly water-soluble investigational compound (RP 69698, Leukotriene B<sub>4</sub> antagonist) with solid dispersion formulations in PEG 3350, Transcutol and Labrasol. They achieved about 2-fold improvement over bioavailability observed with an aqueous suspension of the drug in 0.5% methylcellulose. The bioavailability of ritonavir (Norvir, Abbot), a poorly soluble HIV protease inhibitor, was enhanced by formulation as a solid

dispersion in a mixture of Gelucire 50/13, polysorbate 80 and polyoxyl 35 castor oil [172].

The advantage of solid dispersion, compared with conventional capsule and tablet formulations, is shown schematically in figure 1.22. From conventional capsules and tablets, the dissolution rate is limited by the size of the primary particles formed after the disintegration of dosage forms. In this case, an average particle size of 5  $\mu\text{m}$  is usually the lower limit, although higher particle sizes are preferred for ease of handling, formulation, and manufacturing. On the other hand, if a solid dispersion or a solid solution is used, a portion of the drug dissolves immediately to saturate the gastrointestinal fluid, and the excess drug precipitates out as fine colloidal particles or oily globules of submicron size. During the last three decades following the initial work of Sekiguchi and Obi <sup>[168]</sup> only two products were marketed as solid dispersion; griseofulvin in polyethylene glycol (Gris-PEG, Novartis) and nabilone in povidone (Cesamet, Lilly).

There are certain limitations which hinder the commercial application of solid dispersion systems. These limitations were reviewed by Serrajuddin <sup>[173]</sup>, they involve:

(a) method of preparation, (b) reproducibility of physicochemical properties, (d) dosage form development, (d) scale up of manufacturing processes and (e) physical and chemical stability of drug and vehicle.

The stability issue will only be discussed in this thesis as this project involves producing stable solid self-emulsified lipid systems.

The physical instability of solid dispersions is mainly due to crystallization of drugs. In a solid dispersion prepared by the melt method, a certain fraction of the drug may remain molecularly dispersed, depending on its solubility in the carrier used, thus forming a solid solution. It may however form a supersaturated solution, separate out as an amorphous phase, or crystallize out. The supersaturated and amorphous forms may, in turn, crystallize out on aging. This defeats the purpose of the solid dispersion systems which are designed to improve the dissolution rate and thus enhance absorption, see figure 1.22. Chiou <sup>[174]</sup> reported that griseofulvin precipitated out in an amorphous form in a griseofulvin-PEG 6000 solid dispersion during the time of its preparation. The amorphous material crystallized out on aging, except when the drug concentration in the dispersion was 5% or less. Ford and Rubinstein <sup>[175]</sup> attributed similar crystallization as the cause for a decrease in dissolution rate of drug from

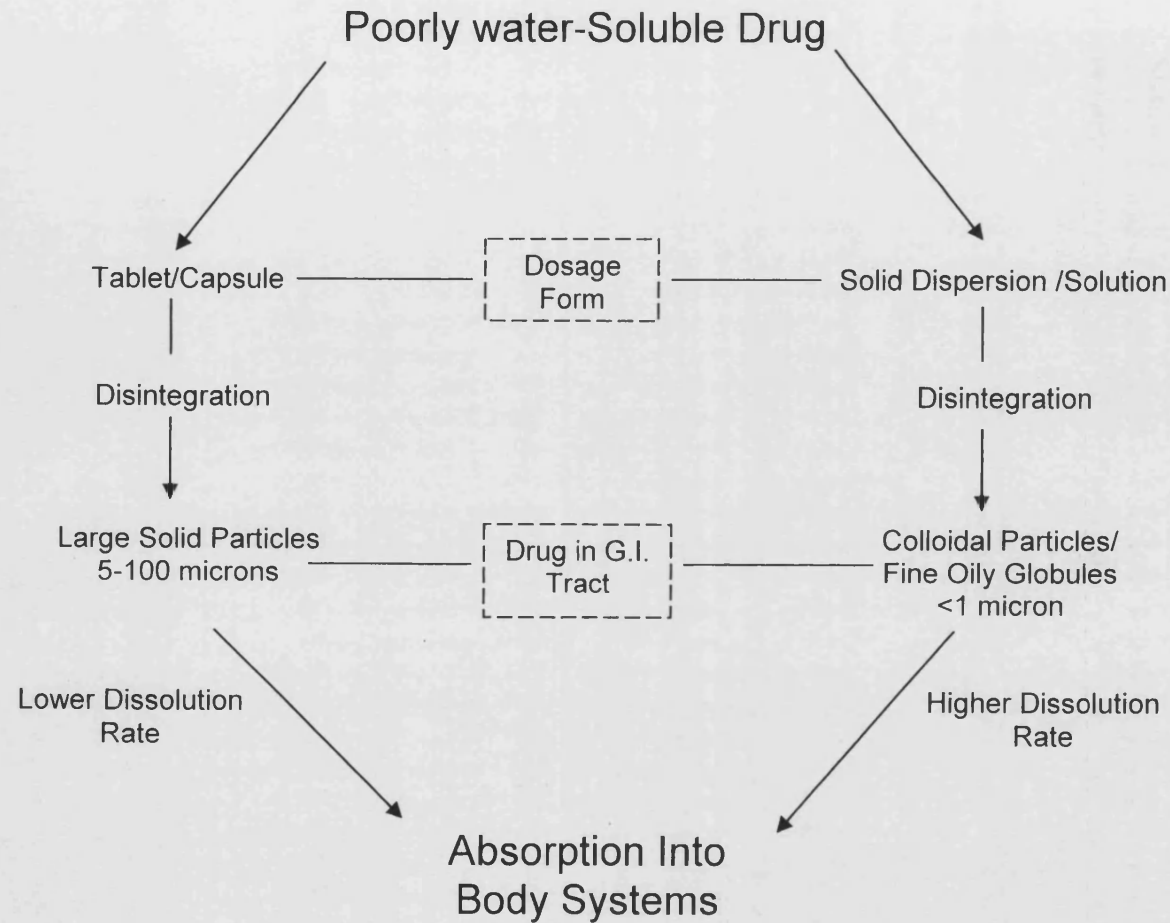


Figure 1.22: schematic representation of the bioavailability of enhancement of a poorly water-soluble drug by solid Dispersion compared with conventional tablet or capsule (adapted from Ref. 173).

indomethacin-PEG 6000 solid dispersion with time. The decrease was greater for a higher drug concentration because a larger fraction of drug crystallized out. In another study, Suzuki and Sunada <sup>[176]</sup> observed that on exposure of a nifedipine-nicotinamide-hydroxypropylmethycellulose (HPMC) solid dispersion to 60% RH at 30 °C or 75% RH at 40 °C for 1 month, nifedipine converted from the amorphous to the crystalline state, thus lowering the dissolution rate of nifedipine drastically. Stabilization of drugs in amorphous forms in solid dispersions is an active area of research in the pharmaceutical field.

## Chapter 2

### Materials and Methods

#### 2.1. Materials Description

##### 2.1.1. Surfactants

##### 2.1.1.1. Tagat<sup>®</sup> Surfactants (Polyoxyethylene glycerol fatty acid esters)

The Tagat products are non-ionic surfactants and can be used on their own or in combination with anionic or cationic surfactants, with which they are compatible. The main field of application is the production of liquid and paste-like emulsions, for which blends of Tagat products are recommended. The Tagat products are used e.g. for paraffin emulsions, microwax emulsions, polyethylene emulsions, wax-paraffin-solvent emulsions, mineral oil emulsions, emulsions of all kinds of solvents, textile auxiliary emulsions, emulsions of paint binding agents and synthetic resins. Some of Tagat products have strongly hydrotropic properties and therefore excellent solubilisers.

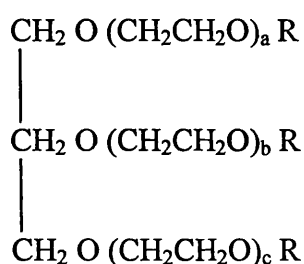
**Table 2.1: TAGAT<sup>®</sup> Products manufactured by Goldschmidt, Germany**

Product name	Chemical name
TAGAT <sup>®</sup> L	PEG-30- Glyceryl Laurate
TAGAT <sup>®</sup> L 2	PEG-20- Glyceryl Laurate
TAGAT <sup>®</sup> O 2	PEG-20- Glyceryl Monooleate
TAGAT <sup>®</sup> R 40	PEG-40- Hydrogenated Caster Oil
TAGAT <sup>®</sup> R 60	PEG-60- Hydrogenated Caster Oil
TAGAT <sup>®</sup> S	PEG-30- Glyceryl Stearate
TAGAT <sup>®</sup> S 2	PEG-30- Glycerl Stearate
TAGAT <sup>®</sup> TO	PEG-25- Glyceryl Trioleate



Tagat<sup>®</sup> TO

Tagat<sup>®</sup> TO (polyoxyethylene-(25)-glyceryl trioleate) was mainly used in this work; it is lipophilic surfactant with an HLB value of 11.3. According to the manufacturer (Goldschmidt Chemical, Germany), Tagat<sup>®</sup> TO is soluble in oil but not in water rather it is dispersible *i.e.* it does not form micelles when mixed with water. It is produced by the esterefication of ethoxylated glycerol with oleic acid. Initially a known weight of ethylene oxide is reacted with glycerol to produce polyethylene glycol glyceryl ether. Esterification is then performed using commercial grade oleic acid, obtained from the hydrolysis and fractionation of olive oil. The resulting compound, Tagat TO, contains an average of 25 ethoxy residues per molecule (see chemical structure in figure 2.1).

Figure 2.1

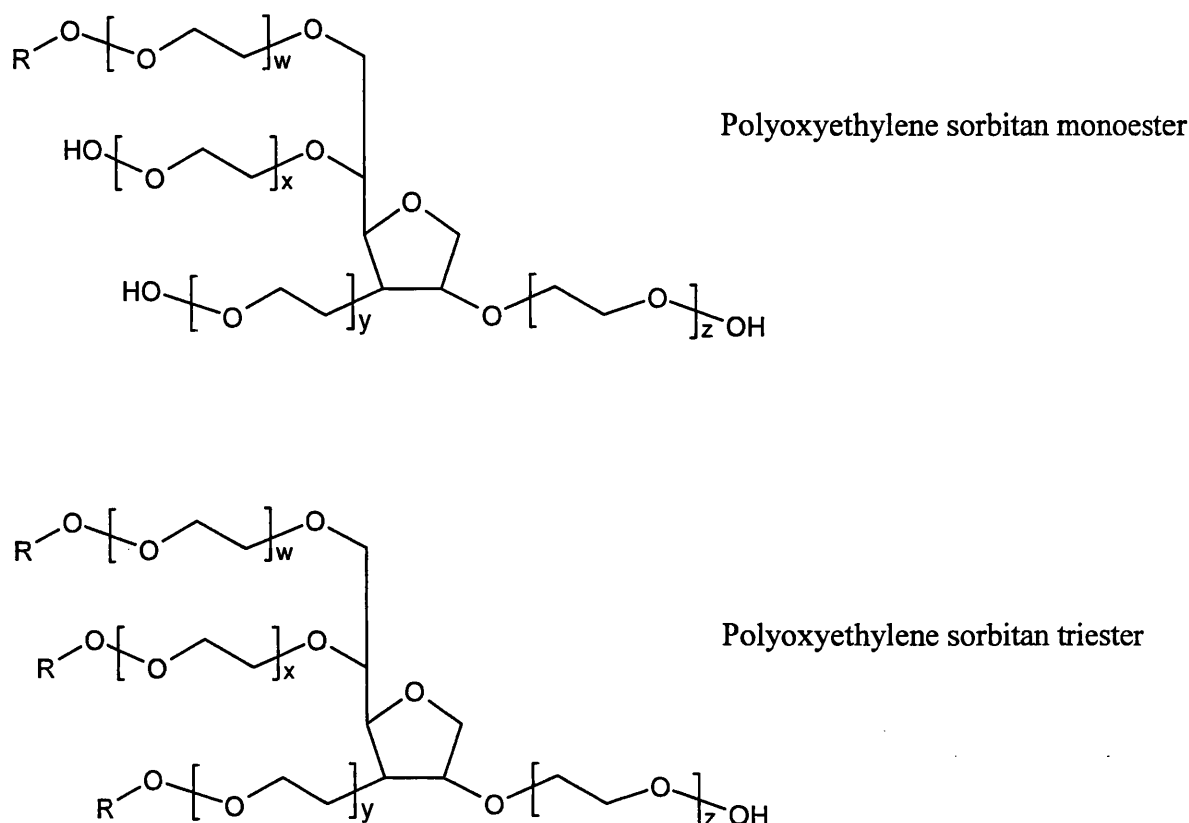
Chemical structure of Tagat TO surfactant, where  $a+b+c = 25$ . R is principally  $\text{CH}_3(\text{CH}_2)_7\text{CH}=\text{CH}(\text{CH}_2)_7\text{COOH}$

Tagat O2

Tagat O<sub>2</sub> is another product of Tagat<sup>®</sup> manufactured and supplied by Goldschmidt Chemical, and was also used in this study. Tagat O<sub>2</sub> (polyoxyethylene-(20)-glyceryl monooleate), contains an average of 20 ethoxy residues per molecule. It is classified as a non-ionic hydrophilic surfactant with an HLB value of 15. According to the manufacturer, Tagat<sup>®</sup> O<sub>2</sub> is not soluble in vegetable oil, soluble in cosmetic alcohol also soluble in water.

### 2.1.1.2. Crillets

Crillets are polyethoxylated partial esters of sorbitan, the cyclic ether/alcohol produced by the dehydration of the hexahydric alcohol, sorbitol. They have the following general formula:



**Figure 2.2**

Chemical structure of Crillets surfactants (Polyoxyethylene Sorbitan Esters), where R is the alkyl group of a fatty acid and  $(w+x+y+z)$  is the total number of moles of ethylene oxide.

Crillets are hydrophilic in nature and are soluble in water and dilute solutions of electrolytes. The solubility of Crillits in aqueous solutions increases with the degree of ethoxylation. For a fixed degree of ethoxylation, aqueous solubility decreases as the number of ester groupings increase (*i.e.* HLB is decreased). For a fixed degree of ethoxylation and esterification aqueous solubility decreases as the molecular weight

of the fatty acid increases Crillet products are manufactured and supplied by Croda Chemicals.

#### Crillet 4

Crillet 4 (Polyoxyethylene-(20)-sorbitan monooleate), also known as Tween 80 or polysorbate 80, was mainly used in this work. It contains an average of 20 ethoxy residues per molecule ( $w+x+y+z = 20$ , figure 2.2), and an HLB value of 15.

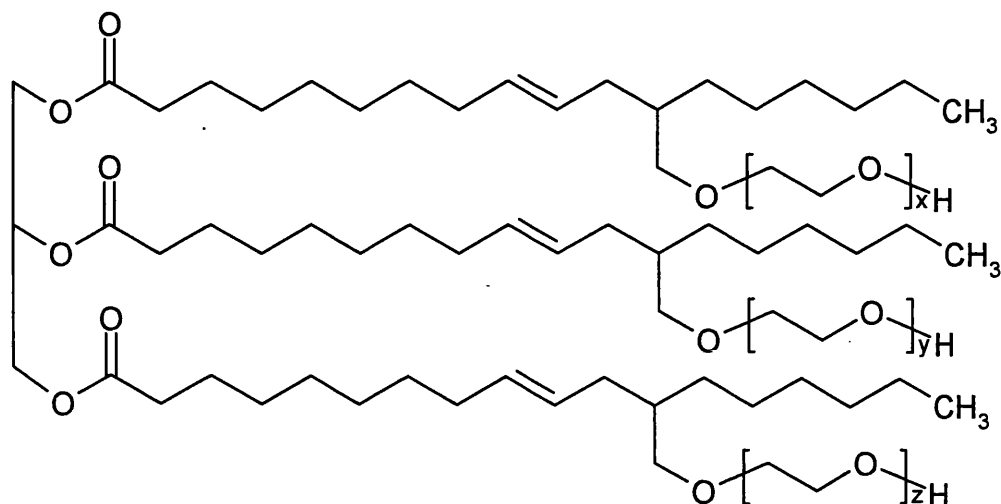
Crillet 4 is a clear, pale yellow to amber liquid with a faint but characteristic fatty odour. In cosmetic and pharmaceutical preparations Crillet 4 functions as an emulsifier and dispersant often in conjunction with other appropriate surfactants; it is an excellent emulsifier for solvents, waxes, silicones, etc. Crillet 4 is also used as a solubiliser, giving clear solutions of oil in water.

#### Crillet 1

Crillet 1 (Polyoxyethylene-(20)-sorbitan monolaurate), also known as Tween 20 or polysorbate 20 was also used in this work. It contains an average of 20 ethoxy residues per molecule ( $w+x+y+z = 20$ , figure 2.2), and an HLB value of 16.7. It is a fully saturated liquid ester with low odour and pale colour. It is the preferred solubiliser for essential oils and perfumes in cosmetic systems. In pharmaceutical preparations, Crillet 1 acts as an emulsifier and dispersant for medicaments such as menthol, phenol, etc, which otherwise separate. It is also used in conjunction with other surfactants as a primary emulsifier in cosmetic creams and lotions.

#### **2.1.1.3. Polyoxyethylene Caster Oil Derivatives**

Polyoxyethylene caster oil derivatives are a series of nonionic surfactants obtained by reacting varying amounts of ethylene oxide with either caster oil or hydrogenated caster oil. They are used in oral, topical and parenteral pharmaceutical formulations, and also in cosmetics and animal feeds. Several different types of material are commercially available the best known being the Cremophors (BASF Corporation). Amongst of these, Cremophor RH40 and Cremophor EL were mainly used in this work. The general chemical structure for cremophors is presented below in figure 2.3.



**Figure 2.3**

Chemical structure of Cremophors, in the case of hydrogenated products the double bond is saturated by hydrogenation, resulting in a more chemically stable structure with superior color and odour. The number of moles of ethylene oxide reacted per mole of castor oil is the sum of  $x$ ,  $y$  and  $z$  which is expressed as the ethoxylation number.

#### Cremophor RH 40

Cremophor RH 40 (polyoxyethylene-(40)-hydrogenated castor oil) is a hydrophilic non-ionic solubilizer and emulsifying agent obtained by reacting 45 moles of ethylene oxide with 1 mole of hydrogenated castor oil. The main constituent of Cremophore RH40 is glycerol polyethylene glycol oxystearate, which, together with fatty acid glycerol polyglycol esters, forms the hydrophobic part of the product. The hydrophilic part consists of polyethylene glycols and glycerol ethoxylate. Cremophor RH 40 is a white to yellowish thin paste with a melting point of  $\approx 40^\circ\text{C}$  and an HLB value between 14 and 16. Cremophor RH 40 forms clear solutions in water, ethanol, 2-propanol and other solvents. In contrast to the anionic emulsifying agents, the solubility of Cremophors in water decreases with rising temperature. Thus, aqueous solutions become turbid at certain temperature.

In aqueous alcoholic or completely aqueous solutions, Cremophor RH 40 can be used to solubilise vitamins (A, D, E and K) essential oils and certain drugs for oral and topical administration. The fact that Cremophor RH 40 has a very little taste or odor,

is an asset for such applications. Cremophor RH 40 is also suitable as an emulsifying agent. It can emulsify a wide range of hydrophobic substances such as, fatty acids, fatty alcohols and drugs.

### Cremophor EL

Cremophor EL (polyoxyethylene-(35)-caster oil) is a non-ionic solubilizer and emulsifier obtained by reacting ethylene oxide with castor oil in a molar ratio of 35 moles to 1 mole. The main component of Cremophor EL is glycerol-polyethylene glycol ricinoleate, which, together with fatty acid esters of polyethyleneglycol, represents the hydrophobic part of the product. The smaller hydrophilic part consists of polyethylene glycols and ethoxylated glycerol. Cremophor EL is a pale yellow, oily liquid that is clear at temperatures above 26°C. It has a slight but characteristic odor and hence, Cremophor RH 40 may be used in preference to Cremophor EL in oral formulations since it is almost tasteless. Nonetheless, the inherent odor of Cremophor EL can best be masked in many cases with banana aroma. Cremophor EL forms clear solutions in water ethyl alcohol, n-propyl alcohol and other solvents. The hydrophilic-lipophilic balance (HLB) lies between 12 and 14.

Cremophor EL is mainly used as an emulsifying and solubilizing agent, and is particularly suitable for the production of aqueous liquid preparations (alcoholic and non-alcoholic aqueous solutions) containing volatile oils, fat-soluble vitamins and other hydrophobic substances. Cremophor EL is also used in cosmetics industry preferentially for solubilizing perfume oils and for emulsifying fatty substances, organic solvents, and additives. A special application of Cremophor EL is the production of cod-liver oil emulsions in veterinary medicine.

## **2.1.2. Oils**

### **2.1.2.1. Medium Chain Triglycerides (Miglyol®)**

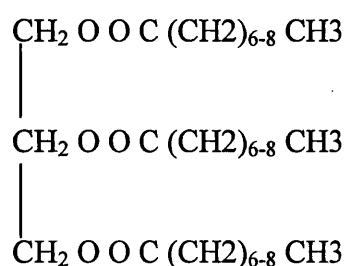
Miglyol natural oils (produced by Condea Chemie GmbH, Hüls) are clear, slightly yellowish esters of the saturated coconut and palmkernel fatty acids caprylic and capric acid with glycerol or propylene glycol as in Miglyol 840. There are different

types of Miglyol<sup>®</sup> products according to the composition of the fatty acid fraction (see table 2.2).

**Table 2.2: Carboxylic acids composition of Miglyol<sup>®</sup> products**

Amount of carboxylic acids in %	Miglyol				
	810	812	818	829	840
Caproic acid (C <sub>6:0</sub> )	Max. 2	Max. 2	Max. 2	Max.2	Max. 2
Caprylic acid (C <sub>8:0</sub> )	65-80	50-65	45-65	45-55	65-80
Capric acid (C <sub>10:0</sub> )	20-35	30-45	30-45	30-40	20-35
Lauric acid (C <sub>12:0</sub> )	Max. 2	Max. 2	Max. 3	Max. 3	Max.2
Myristic acid (C <sub>14:0</sub> )	Max. 1	Max. 1	Max. 1	Max. 1	Max. 1
Linoleic acid (C <sub>18:2</sub> )	—	—	2-5	—	—
Succinic acid	—	—	—	15-20	—

Miglyol 812, a saturated medium chain triglyceride, was used in the majority of the formulations. It is produced by the hydrolysis of coconut oil followed by fractionation to yield the required fatty acids which are then re-esterified with glycerol. The general structure of Miglyol 812 is shown in figure 2.4. The composition of the fatty acid fraction is given in table 2.2.



**Figure 2.4**

Chemical structure of Miglyol 812

Medium chain triglycerides (MCTs) have been used in a variety of pharmaceutical formulations including oral, parenteral and topical preparations. In oral formulations, MCTs are used as the base for the preparation of oral emulsions, solutions or suspensions of drugs unstable or insoluble in aqueous media. They have also been

investigated as intestinal absorption enhancers and have additionally been used as filler in capsules and sugar-coated tablets, and as a lubricant or anti-adhesion agent in tablets. In parenteral formulations, MCTs have similarly been used in the production of emulsions, solutions or suspensions intended for intravenous administration. Medium chain triglycerides have been particularly investigated for their use in total parenteral nutrition (TPN) regimens in combination with long chain triglycerides. In cosmetics and topical pharmaceutical preparations, MCTs are used as a component of ointments, creams and liquid emulsions.

#### **2.1.2.2. Partial Glycerides (Imwitor<sup>®</sup>)**

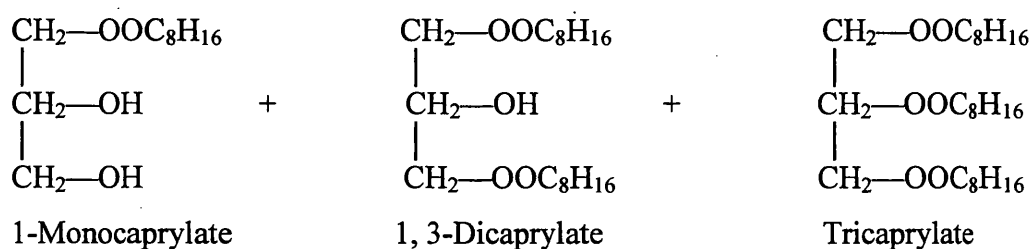
Partial glycerides are usually mixtures of monoesters, diesters and triesters containing small proportions of unesterified glycerol. Some types have very high monoester content such as Imwitor 308, 312 and 191. The fatty acids used are derived exclusively from natural fats and oils, and have a chain length of 8-18 carbon atoms. The partial glycerides are produced either by transesterification of fats with an excess of glycerol or by controlled esterification of fatty acid fractions, followed by refining processes. Both methods yield products containing approximately 40-60% monoglycerides. Monoglyceride contents of over 90% can be obtained by subsequent molecular distillation.

The degree of surface activity (hydrophilic or lipophilic nature) is determined by the number of free unesterified hydroxyl groups and the length of the fatty acids. Partial glycerides with high monoglyceride content have high HLB values (i.e. more polar) and may form liquid crystalline states, micelles and vesicles in the presence of water. HÜLS partial glycerides are widely used in the pharmaceutical, cosmetic and nutritional fields. They function as emulsifiers, co-emulsifiers for o/w creams and lotions, solubilisers, dispersants, plasticizers in tablet coating, lubricants, skin and mucous membrane protecting agents and absorption promoters for active ingredients which are not soluble on their own in oral drug forms. Monoglycerides of the C8-C14 fatty acids can also act as antimicrobial substances that help to prevent mycotic infections.

Imwitor 988 and 308 were mainly used in the majority of formulations investigated in this study, see table 2.3 for fatty acid composition and figure 2.5 for chemical structure.

**Table 2.3: Some characteristic values of Imwitor 308 and 988**

	Imwitor 308 (Glycerol mono-caprylate)	Imwitor 988 (Glycerol mono-/di-caprylate)
<b>Typical Composition</b>		
Free Glycerol	1	1
Monoglycerides	90	50
Diglycerides	7	40
Triglycerides	1	6
Water content	Max. 1	Max. 2
<b>Properties</b>		
Appearance	White Crystalline	Colorless Semisolid
Melting point (°C)	30-34	Liq. at 25
HLB value	6	4-6



**Figure 2.5**

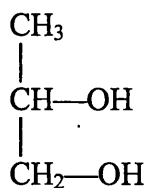
Chemical structure of Imwitor 988 and 308.

### 2.1.3. Co-Solvents Used in Lipid Formulations

#### Propylene Glycol:

1,2,-Propanediol, molecular weight = 400, obtained from Sigma, UK.

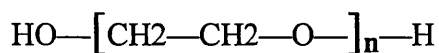


Figure 2.6

Chemical structure of Propylene glycol

Polyethylene Glycol 400:

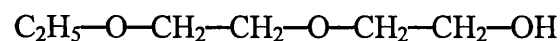
Poly(oxy-1,2-ethandiyl), molecular weight = 400, obtained from Sigma, UK.

Figure 2.7

Chemical structure of Polyethylene glycol, n = No of oxyethylene units

Transcutol P® (Ethoxydiglycerol):

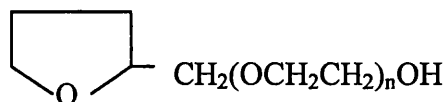
Transcutol P® (diethylene glycol monoethyl ether or Ethoxydiglycerol) is manufactured by Gattefossé Corporation from raw materials of petrochemical origin; it has a molecular weight of 134.7. Transcutol offers superior cutaneous tolerance and excellent solubilization of many drugs. It is soluble in both oil and water, therefore, it represents the perfect bridge between the oil and water phase. It has several areas of applications: vaginal douches, oral, topical and injection formulations.

Figure 2.8

Chemical structure of Transcutol

Glycofurol®

Glycofurol (α-Tetrahydro furanyl -w-hydroxy-poly (oxyethylene)) is used as a solvent mainly in parenteral products for intravenous or intramuscular injection in concentrations up to 50% v/v. It is manufactured and supplied by Roche, Switzerland.

**Figure 2.9**

Chemical structure of Glycofurol,  $n = 1-2$ .

#### 2.1.4. Emulsification Solutions

MiliQ water

Simulated Gastric Fluid (SGF)

This is a media used to simulate the composition of stomach at fasted state, see table 2.3 for composition.

**Table 2.4: Composition of the physiological media used to simulate the stomach at fasted state**

Simulated Gastric Fluid (SGF)				
NaCl	Pepsin (800-2500 u/mg)	HCl	Water (qs)	pH
2 gm	3.2 gm	7 ml	1000 ml	1.2

Pepsin is insoluble in this media and thus forms a turbid solution which will make particle sizing rather difficult. Consequently, SGF was freshly prepared or obtained from Sigma without Pepsin.

FaSSIF and FeSSIF

Fasted state simulated intestinal fluid (FaSSIF) and fed state simulated intestinal fluid (FeSSIF) are suitable media for simulating the composition of the proximal small intestine at fasting and fed states, respectively. See table 2.4 for composition.

FaSSIF and FeSSIF were prepared by first making the micellar solution which consists of bile salt (Na Taurcholate) and Lecithin (L- $\alpha$  phosphatidylcholin) at a ratio of 3:1. To prepare the mixed micellar solution appropriate quantities of bile salt and lecithin (see table 2.4) were dissolved in 20 ml of solvent consisted of methanol and chloroform at a ratio of 2:3. The resultant clear solution was placed in a 500 ml Büchi flask and the solvent drawn off using rotary evaporator (Büchi Rotavapor R110). The flask was then attached to a vacuum line overnight to ensure removal of any remaining chloroform. The clear film of bile salt and lecithin formed on the flask surface was subsequently dissolved in the appropriate buffer (see table below) to prepare either FaSSIF or FeSSIF at the required concentration of bile salt / lecithin.

**Table 2.5: Composition of two physiological media used to simulate fasted state and fed state intestinal conditions.**

Fasted State Simulated Intestinal Fluid (FaSSIF)		Fed State Simulated Intestinal Fluid (FeSSIF)	
pH	6.5	pH	5
Osmolarity	270 $\pm$ 10 m Osmol	Osmolarity	635 $\pm$ 10 m Osmol
Na Taurcholate	3 mM	Na Taurcholate	15 mM
Lecithin	0.75 mM	Lecithin	3.75 mM
KH <sub>2</sub> PO <sub>4</sub>	3.9g	Acetic Acid	8.65g
KCL	7.7g	KCL	15.2g
NaOH	qs pH 6.5	NaOH	qs pH 5
Mili1 Q water	qs 1 liter	Mili Q water	qs 1 liter

Na Taurcholate, Lecithin and KCL were obtained from Sigma, and KH<sub>2</sub>PO<sub>4</sub> from BDH Chemicals.

#### 2.1.5. Solvents Used for Assays

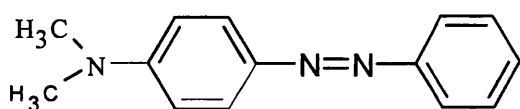
Ethanol 96% w/w GPR, BDH Chemicals

Methanol 96% v/v GPR, BDH Chemicals

### 2.1.6. Model Drug

Dimethyl aminoazobenzene was used in this study as a model drug of ‘poorly soluble’ weak base (log P of 4.52, pKa of 3.226 and  $S_0 = 1.33 \mu\text{g/ml}$ ), see figure 2.10 for chemical structure. It occurs as yellow crystalline leaflets. It is insoluble in water and soluble in alcohol, benzene, chloroform, ether, petroleum ether, strong minerals acids, oils and very soluble in pyridine.

Dimethyl aminoazobenzene was previously used to color polishes, polystyrene and soap. It was also used as chemical indicator for free hydrogen chloride in gastric juice and as pH indicator. Dimethyl aminoazobenzene is not currently produced or used commercially in the United States due its environmental and health hazardous effects. Therefore, strict procedures were followed in accordance with the chemical safety sheets in the handling or disposal of this material whenever it was used.



**Figure 2.10**

Chemical structure of p-Dimethyl Aminoazobenzene also known as Dimethyl Yellow (DMY) which was used as a model drug for poorly water-soluble weak electrolyte compounds

## 2.2. Methods

### 2.2.1. Ternary Phase Diagrams

Regions of mutual solubility of various lipid formulations with wide range of surfactants that represent different HLB values were determined using ternary phase diagrams. Miscibility diagrams of Miglyol 812, Imwitor 988 and various surfactants (Tagat TO, Tagat O2, Crillet 4, Crillet 1, Cremophor RH 40 and Cremophor EL) were constructed. Each of the axes on the diagram represents the percentage contributes to the formulation by each of the three components (Miglyol 812, Imwitor 988 and a surfactant). Formulations of five grams which represent various percentages of Miglyol 812, Imwitor 988 and a surfactant on the ternary phase diagrams, were

weighed in 20 g screw-capped vials (tops were rapped with cling film before caps were screwed on). Mixtures were placed in a water bath at 50 °C for 2 minutes before the three components were thoroughly vortexed. Mixtures were then kept for 24-48 hours in an oven set up at 25°C before visual assessment. Mixtures which formed a continuous single phase were classified as miscible formulations. Samples that displayed two or more phases were described as immiscible systems.

### 2.2.2. Self-Emulsification

Mixtures of oil and surfactant were produced by accurately weighing ingredients into screw-capped glass vials with tight closures followed by vortexing. Different proportions (w/w) of Miglyol 812/Imwitor 988 (10:0, 9:1, 8:2, 7:3, 6:4, 5:5, 3:7, 1:9 and 0:10) containing between 5 and 60% w/w surfactant were weighed first in the glass vials, tops were then rapped with cling film before caps were screwed on. The glass vials were held at 50 °C in a thermostated water bath held for 2 minute before lipid mixtures were thoroughly vortexed. Lipid formulations were then left to equilibrate over night in an oven set up at 25°C. Emulsions were prepared under conditions of gentle agitation at a controlled temperature of 37°C. Self-emulsifiable mixtures (1gm) were introduced into 100ml of Mili Q water in a 500-ml glass beaker held at 37°C in a thermostated water bath. All materials were pre-equilibrated to the appropriate temperature. Emulsification under agitation conditions considered to be a reasonable simulation of the *in vivo* situation was carried out. Agitation was provided by gentle shaking on a mechanical shaker at 100 oscillations per min for 15 minutes. Systems which appeared to emulsify efficiently were studied further by particle size analysis.

#### 2.2.2.1. Effect of Emulsification Temperature

Emulsions formed by mixtures of Miglyol 812/Imwitor 988 (10:0, 7:3, 6:4 and 5:5) containing 15% or 30% w/w Tagat TO were investigated over temperature range between 5 and 60°C. Emulsions were prepared under conditions of gentle agitation at the required temperature as described earlier. Samples were then removed after 15

minutes and left to equilibrate at 25°C in thermostatted water bath before particle sizing.

#### **2.2.2.2. Effect of Emulsification Media**

Self-emulsifiable mixtures (1gm) of different oil systems without the model drug were introduced into 100ml of SGF in a 500-ml glass beaker held at 37°C in a thermostated water bath and emulsified for 15 min as described earlier before particle sizing. Selected oil systems in the presence of DMY were also emulsified as described above in 100m of either FaSSIF or FeSSIF.

#### **2.2.3. Analysis of Mean Emulsion Droplet Diameter (MEDD)**

A wide range of techniques are available to measure the particle size of two-phase disperse systems. However, when considering the measurement of droplets of emulsions, the number of methods which can be applied is limited. The main techniques which can be used are; image formation, scattering or diffraction of radiation, and relative motion between particles and fluids.

Electron microscopy can be used to measure the submicron particle size range yet sample preparation can result in changes in the suspended droplets. Nonetheless, particle size data can be obtained however, the cost and time involved makes this method inappropriate for processing large numbers of samples. There are a number of light scattering methods available for particle size analysis in the submicron range. Photon correlation spectroscopy (PCS) which is based on dynamic light scattering theory was used in this study. PCS is used to determine the size of emulsions droplets, particles in suspension and molecules in solution with diameters in the range 3nm to 3µm. The theoretical consideration of particle size measurement by PCS is based on the behavior of submicron particles to forces of Brownian motion. The random Brownian movement of spherical particles in a dispersion medium at a constant temperature and viscosity is a function of particle size. For this study Malvern model Lo-C photon correlation spectrometer was used, the components of the instrument are shown in Figure 2.11.

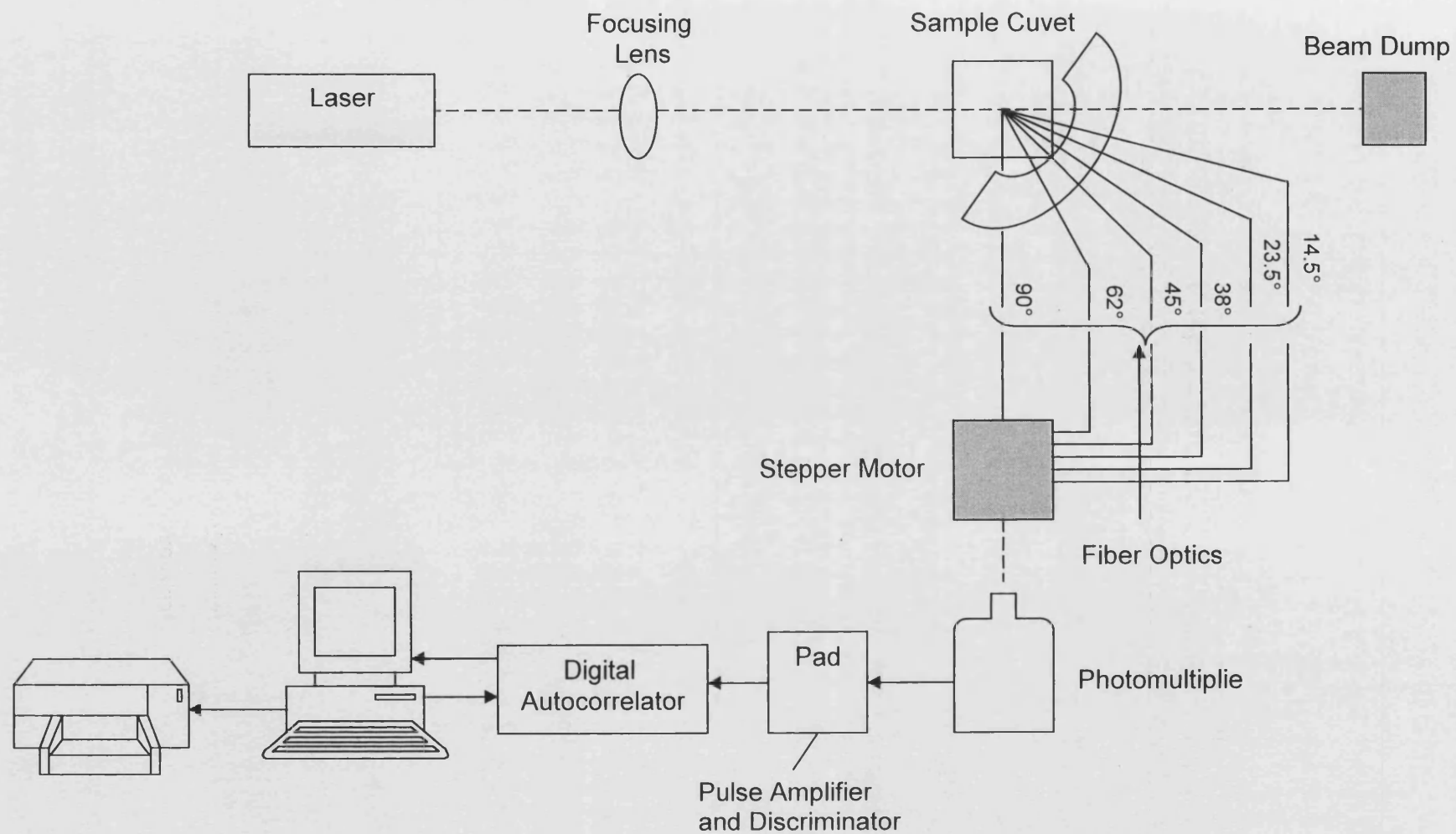


Figure 2.11

The system components of the Autosizer Lo-C Photon Correlation Spectrometer (PCS); the apparatus is set up at a fixed angle of 90°.

Laser diffraction spectrometer (Malvern Mastersizer X) was used also used to analyze self-emulsified systems containing emulsion droplets larger than  $1\mu\text{m}$ . The Fraunhofer diffraction theory which governs this technique points out that the intensity of the light scattered in a forward direction is proportional to the particle size and that the size of the diffraction pattern (scattering angle) is inversely proportional to the particle size. The Malvern equipment monitors the dynamic diffraction pattern using a 50 element concentric ring detector. The equipment was used with small volume cell and with a 45mm lens before the photodetector, allowing measurement of emulsion droplets in the  $0.5\mu\text{m}$  to  $80\mu\text{m}$ . The basic components of the machine are shown in Figure 2.12.

The two techniques mentioned above were used to measure the MEDD of the self-emulsified systems (with appropriate dilution). Low angle laser light diffraction was used for emulsions with droplet distributions above  $1\mu\text{m}$ . Samples were analyzed immediately after preparation. Samples of submicron dispersions were analyzed immediately using PCS. For both techniques experiments were performed in triplicate and the size distributions of the resultant emulsions were obtained using the volume-average diameters  $D(v, 0.5)$ . MEDD values are expressed as mean values of all data  $\pm$  standard error.

#### **2.2.4. Equilibrium Phase Studies**

The equilibrium phase behavior of both ternary oil-nonionic surfactant-water mixtures and binary nonionic surfactant-water mixtures were studied in two ways.

##### **2.2.4.1. Static Compositions**

A series of ternary phase compositions were made up by weight in screw-capped glass vials. For construction of the whole ternary phase diagram, for example the Miglyol-Tagat TO-water system, phase composition changes were made at 5%w/w intervals. Additional compositions were made at 2.5%w/w intervals where phase boundaries were observed. The various ternary and binary compositions were heated to  $70^\circ\text{C}$  in a thermostatically controlled water bath for 15 minutes with intermittent



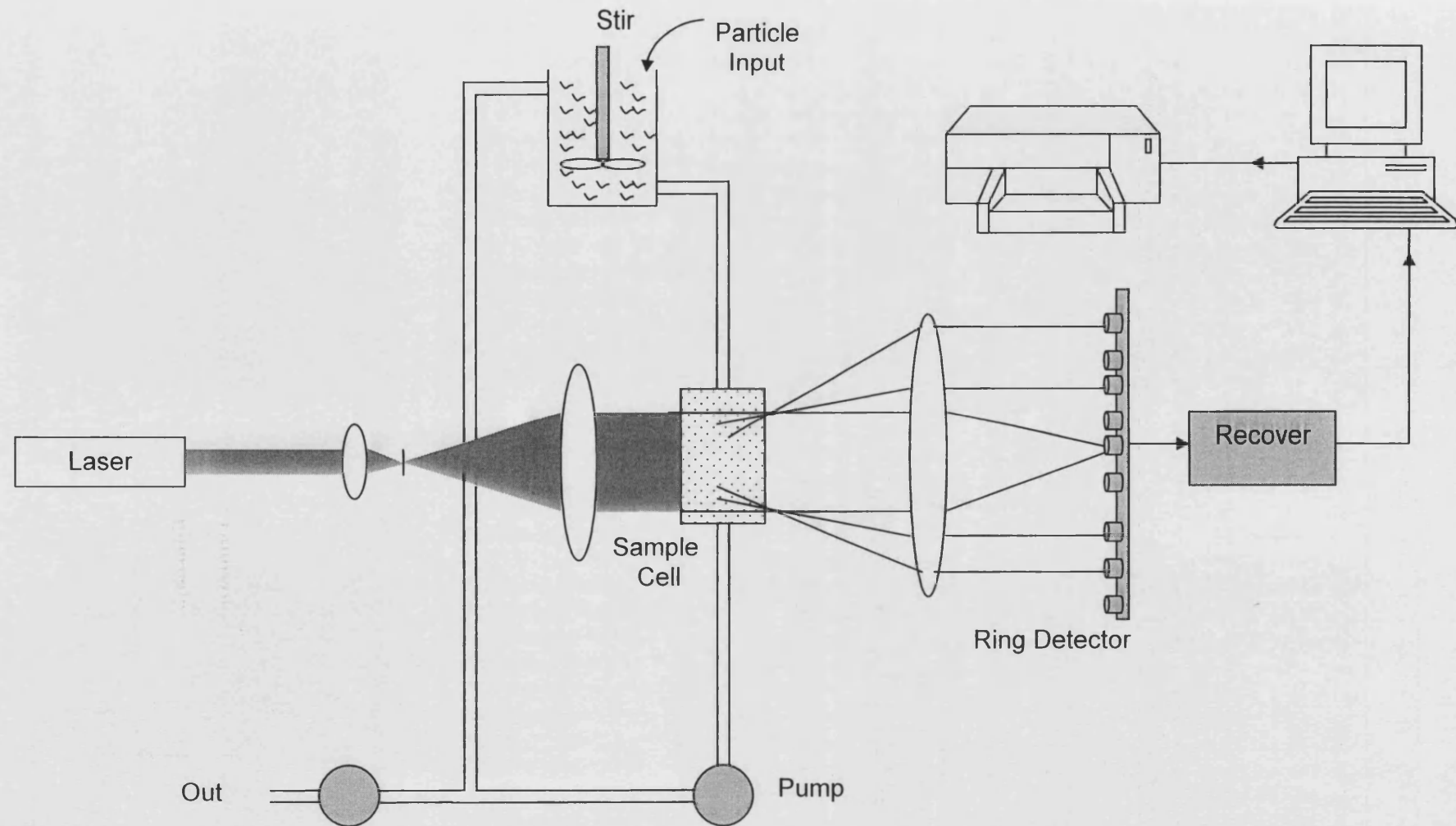


Figure 2.12:  
The system components of the Malvern Mastersizer X Laser Diffraction Spectrometer.

mixing using a Fisons orbital vortex mixer until homogeneity was achieved. The mixtures were then allowed to cool to 25°C and left undisturbed for 24 hours for phase identification by visual observation using a crossed polarised viewer.

#### **2.2.4.2. Dynamic Compositions**

For this method the weighed amounts and therefore the ratios of oil and nonionic surfactant in each mixture were constant, the only difference is the water content. Phase studies were performed using approximately 2 g samples of oil-surfactant mixtures diluted by sequential increasing of water content. Formulations of interest, Miglyol 812/Imwitor 988 at ratios 10:0, 9:1, 8:2, 7:3 and 0:10 containing 15% or 30% w/w Tagat TO were weighed into narrow bore glass ampoules. MiliQ water was then sequentially added to the oil mixtures at 0.5 % w/w intervals, ampoules were then sealed using an Adelphi ampoule sealer. The ampoules were then heated to 70°C for 15 min to facilitate mixing using a votrexer. Phase boundaries were determined to  $\pm 0.5\%$  water. The ternary mixtures were equilibrated to the temperature of interest ( $4-65 \pm 0.1^\circ\text{C}$ ) for two hours and then thoroughly mixed for 5 min using a Fisons orbital whirlmixer. The ampoules were then returned to the thermostatically controlled water bath and left undisturbed for 24 hours before identification of the phase type using a crossed polarised viewer. In this study emphasis was placed on approximate identification of the phase boundary for solubilization of water in the oil-surfactant mixtures and the presence of liquid crystalline phases within the mixtures.

#### **2.2.5. Solubility of the Model Drug in Lipid Excipients and Selected Lipid Formulations**

The model drug (Dimethyl Yellow) was added in excess ( $\approx 1\text{g}$ ) to either lipid excipients or to lipid formulations that represent Type I, II, IIIA and IIIB (see table 2.5). Lipid suspensions were then vortexed for 3 minutes and then stored for 24 hours in a controlled temperature oven at 25°C to reach equilibrium (samples were vortexed in between). One of the formulas (Miglyol 812 in excess of DMY) was kept for 1, 2, and 3 days to investigate the time needed to achieve equilibrium state (saturated solution). Oil suspensions were then transferred into 2ml eppendorphs and centrifuged

at maximum speed (13000g) for 10 minutes. The clear saturated oil solution was then removed and assayed analytically by UV spectrophotometry.

**Table 2.6: Selected lipid formulations used to dissolve the model drug to investigate the effect of different oil systems on the solubility of DMY.**

Lipid formulation	Excipients (%w/w)				
	Miglyol 812	Imwitor 988	Tagat TO	Cremophor RH40, EL, or Crillet 4	PEG 400
Type I	70	0	30		0
Type II	49	21	30	0	0
Type IIIA	42, 49, or 49	28, 21, or 21	0	30	0
Type IIIB	0	30	0	30	40

#### 2.2.5.1. UV Assay

An ultraviolet spectroscopic method was developed to determine the concentration of the model drug (DMY) dissolved in the lipid solution. DMY was dissolved in Methanol and analyzed by scanning over a range of wavelengths using a double beam instrument (Perkin-Elmer Lambda 7, UV / VIS Spectrophotometer). Peak wavelength ( $\lambda_{\max}$ ) was determined ( $\lambda = 407\text{nm}$ ) and standard concentration / absorbance curve for DMY was then constructed. The average  $E^{1\%}$  for DMY was calculated from Beer's law by measuring the absorbance of five DMY solutions. Five stock solutions of DMY in Methanol were prepared by dissolving five separate weights (approximately 0.1g) from DMY in 100 ml Methanol for each stock solution. Series of dilutions were carried out for the five stock solutions to enable measuring the absorbance; values between 0.1 and 0.9 were achieved and considered to be acceptable. According to Beer's law which is modified and presented below, the measured absorbance for each solution was divided by its corresponding concentration to determine  $E^{1\%}$  for DMY.

$$E^{1\%} = \frac{A}{L \cdot C} \quad (\text{Eq. 1})$$

Where  $E$  is the absorptivity for a particular absorbing species (in units of  $100\text{ml g}^{-1}\text{cm}^{-1}$ ),  $A$  is the absorption,  $L$  is the length of the path of radiation passing through the sample (1 cm) and  $C$  is the concentration of the absorbing substance in  $\text{g}/100\text{ml}$ .

The calculated  $E^{1\%}$  was the basis to determine the concentration of DMY dissolved in the oil solution. Representative samples (in triplicate) of the oil solutions in the presence of the model drug (DMY) were dissolved in methanol and assayed according to the procedure described above. The measured absorbance which represents each sample was divided by the  $E^{1\%}$  of DMY to determine the concentration of DMY (%w/w) in that sample. Blank solutions were made by dissolving representative samples oil formulations without DMY in methanol.

In the case of determining the solubility of DMY in cosolvent-water systems,  $E^{1\%}$  for DMY was obtained by dissolving particular amounts of DMY in a solvent composed of Methanol/water at a ratio of (50/50). Peak wavelength ( $\lambda_{\text{max}}$ ) was determined and the measured absorbance for each solution was divided by its corresponding concentration.

#### **2.2.5.2. Self-emulsification of Oil Systems Containing Dissolved DMY**

A range of SEDDS representing Type I, II and III were prepared to probe the effect of the inclusion of water-soluble surfactants and cosolvents in the pre-concentrate mixture on precipitation of the drug after dispersion. 1g of each formulation containing approximately 40mg DMY was allowed to emulsify, as explained in 2.3, in either 100 ml of water or simulated intestinal fluid fasted and fed states (FaSSIF and FeSSIF, respectively). Dispersions were then transferred into 150ml bottles and placed on a mechanical shaker set up at 50 oscillations per min to simulate intestinal motility. The solubility of DMY was determined as a function of time using spectrophotometry as described above.

## Chapter 3

# Self-emulsifying and Micro-emulsifying Formulations for Oral Drug Delivery

## Introduction

One of the most persistent challenges faced by the formulation scientists has been to find methods of improving the bioavailability of poorly water-soluble drugs. A drug must almost invariably be in solution within the gastrointestinal (GI) tract before it can cross the GI mucosa thus poor water solubility may lead to incomplete and erratic absorption. Several approaches are being used to overcome these challenges, such as incorporation of the poorly-water soluble active component into inert lipid vehicles such as oils, surfactant dispersions, self-emulsifying formulations and liposomes.

Self-emulsifying drug delivery systems (SEDDS) which are isotropic mixtures of oils and non-ionic surfactants are recently being used for improving lipophilic drug dissolution and absorption <sup>[1-3]</sup>. One characteristic of these systems is their ability to form fine oil-in-water emulsions upon mild agitation when exposed to aqueous media. It is proposed that enhancement in the water solubilisation region ( $L_2$ ) and the formation of interfacial liquid crystal on dilution with water are important to the mechanistic processes. Thus, SEDDS represent an efficient vehicle for the *in vivo* administration of oral delivery of lipophilic drugs with a possible alternative to tablets and capsules, provided however, that the drug has adequate solubility in the oil system. It is also important in order to maximize the rate of absorption and thus bioavailability of oily formulations; one must maintain the drug in solution and avoid crystallization of the drug on dilution in the lumen of the gut.

Self-emulsifying technology for oral delivery use has recently witnessed the introduction of self-emulsifying microemulsion formulations; Neoral (originally marketed as 'Sandimmune<sup>TM</sup>') is one good example of the advances in this technology. However, the design for effective self-micro-emulsifying drug delivery

systems (SMEDDS), using well-defined and pharmaceutically acceptable excipients is still in its early stages <sup>[4]</sup>. Pouton <sup>[5]</sup> has recently suggested a classification system for lipidic formulations based on their dispersions characteristics and the physicochemical characteristics of the formulation components (principally the HLB of the surfactants and % glycerides content (Table 1.5)). Under this system, simple surfactant-free lipid solution formulations are classified as type I. Type II formulations are often referred to as SEDDS which comprise water insoluble components; blends of glycerides and non-ionic surfactants with HLBs less than 12. Type III systems which contain hydrophilic surfactants with HLB >12 and/or water-soluble cosolvents blended with oils. Type III formulations which include water-soluble components are referred to as self-micro-emulsifying systems (SMEDDS) as they can lead to the production of a microemulsion on dispersion in GI tract (particle size  $\approx$  50nm). Type III formulations can be subdivided into type IIIA and type IIIB according to the degree of hydrophilicity of these formulations. Type IIIB systems are more hydrophilic and contain relatively little simple glycerides (<20%).

This chapter is divided into 3 parts:

### Section 3.1

Evaluation of potential self-emulsifying type II vehicles based on comparison of their self-emulsification profiles with the system which was thoroughly investigated by Wakerly <sup>[6]</sup> i.e. Tagat TO-Miglyol 812 system in an attempt to improve the *in vitro* and consequently the *in vivo* performance. It investigates the use of C<sub>8</sub>/C<sub>10</sub> mono/diglycerides (Imwitor 988) as a cosurfactant to enhance the emulsification of Miglyol 812-Tagat TO system. It also elucidates the effects of main factors, namely mixture composition, temperature and media, on controlling the emulsion droplet size distribution of Miglyol 812/Imwitor 988-Tagt TO system. This has been correlated with the equilibrium phase behaviour of these systems on dilution with water in order to provide an insight into the mechanistics of self-emulsification.

### Section 3.2

This part evaluates the self-emulsifying behaviour of type III systems comprising mixed medium chain glycerides (Miglyol 812-Imwitor 988) and wide range of hydrophilic surfactants in an attempt to identify self-emulsifying microemulsion formulations. Non-ionic surfactants with HLB in the range 14 to 16.5 are investigated

amongst these are; Cremophor RH40, Cremophor EL, Polysorbate 80 (Crillet 4), Polysorbate 20 (Crillet 1) and Tagat O2. It verifies the effect of oil component blends on the performance of these systems in order to find an optimum blend of Miglyol 812-Imwitor 988 which produces the finest dispersions. It also correlates the mean emulsion droplet diameter profiles obtained by photon correlation spectrometer (PCS) for systems which have produced microemulsions with their equilibrium phase behaviour on dilution with water.

### Section 3.3

This section investigates how different types of self-emulsifying systems (type II, IIIA and IIIB) influence the fate of dissolved drug after dispersion of the formulation. Dimethyl Yellow (DY) was used as a lipophilic weak base model drug (log P of 4.52 and pKa of 3.226). A range of self-emulsifying systems are investigated to probe the influence of the inclusion of water-soluble surfactants and cosolvents on precipitation of the drug after dispersion.

## **3.1. Emulsification Improvement of Class II SEDDS: Formulation and Mechanistic Perspectives**

### **3.1.1. Results and Discussion**

#### **3.1.1.1. Size Analysis**

Previous studies using binary systems containing medium-chain triglyceride oil and Tagat TO have produced system that exhibits optimum self-emulsifying behaviour at 30°C <sup>[7, 8]</sup>. In contrast to the Tween 85-Miglyol 812 investigated by Pouton <sup>[9]</sup>, the Tagat TO-Miglyol 812 system produced by Wakerly <sup>[8]</sup> was shown to be capable of forming submicron emulsions by self-emulsification. These studies have indicated that emulsification of Miglyol 812-Tagat TO in water using 30% (w/w) surfactant concentration has dramatically decreased the mean emulsion droplet diameter (MEDD).

The effect of incorporating, C<sub>8</sub>/C<sub>10</sub> mono/diglycerides (Imwitor 988) in the binary mixtures of Miglyol 812/Tagat TO on emulsification behaviour has been investigated in this study to establish the optimum requirements for good self-emulsifying systems.

Knowledge of the behaviour of triglyceride, mixed mono/di-glycerides and either hydrophilic or lipophilic surfactant when mixed together is considered to be a preliminary step in selecting a formulation which remains miscible after encapsulation in a soft gelatine capsule. Any phase separation of constituents after the encapsulation stage could result in drug crystallization and hence the addition of drug dissolution stage upon release of the formulation from the capsule. Therefore, a three-component phase diagram was constructed to examine the miscibility of Miglyol 812, Imwitor 988 and Tagat TO, see figure 3.1.1. Each of the axes on the diagram represents the percentage that has contributed to the formulation by each of the three constituents. Therefore, the effect of varying the proportions of constituents on the formulation miscibility can be examined. As illustrated in Figure 3.1.1, Miglyol 812/Imwitor 988-Tagat TO system has produced a whole area of miscibility. This suggests the ability of this system of forming homogenous mixtures at all proportions of its constituents.

In order to investigate the effect of Imwitor 988 on the emulsification behaviour of the binary system (Miglyol 812/Tagat TO), Imwitor 988 was included by varying the ratio fraction of Miglyol 812/Imwitor 988 while gradually increasing the concentration of Tagat TO in each ratio. This inclusion in the binary system is depicted in the drawn lines (A-I) in figure 3.1.1. Lines (A to I) represent increasing ratio fraction of Miglyol 812/Imwitor 988 from 1:9 to 9:1, respectively. All points on each line have constant ratio of Miglyol 812/Imwitor 988 which represents that line diluted with increasing concentration of Tagat TO.

The emulsification profile of Miglyol 812/Tagat TO system was chosen as the starting point to compare the emulsification behaviour that results from the inclusion of Imwitor 988 in the binary mixtures. The particle size-surfactant concentration profiles obtained by laser diffraction analysis for the self-emulsified Miglyol 812/Imwitor 988-Tagat TO mixtures is shown in figure 3.1.2. The self-emulsification profile of Miglyol 812/Tagat TO presents three regions: (1) Surfactant concentration <15% (w/w), region of gross instability with high creaming rate of these lower surfactant



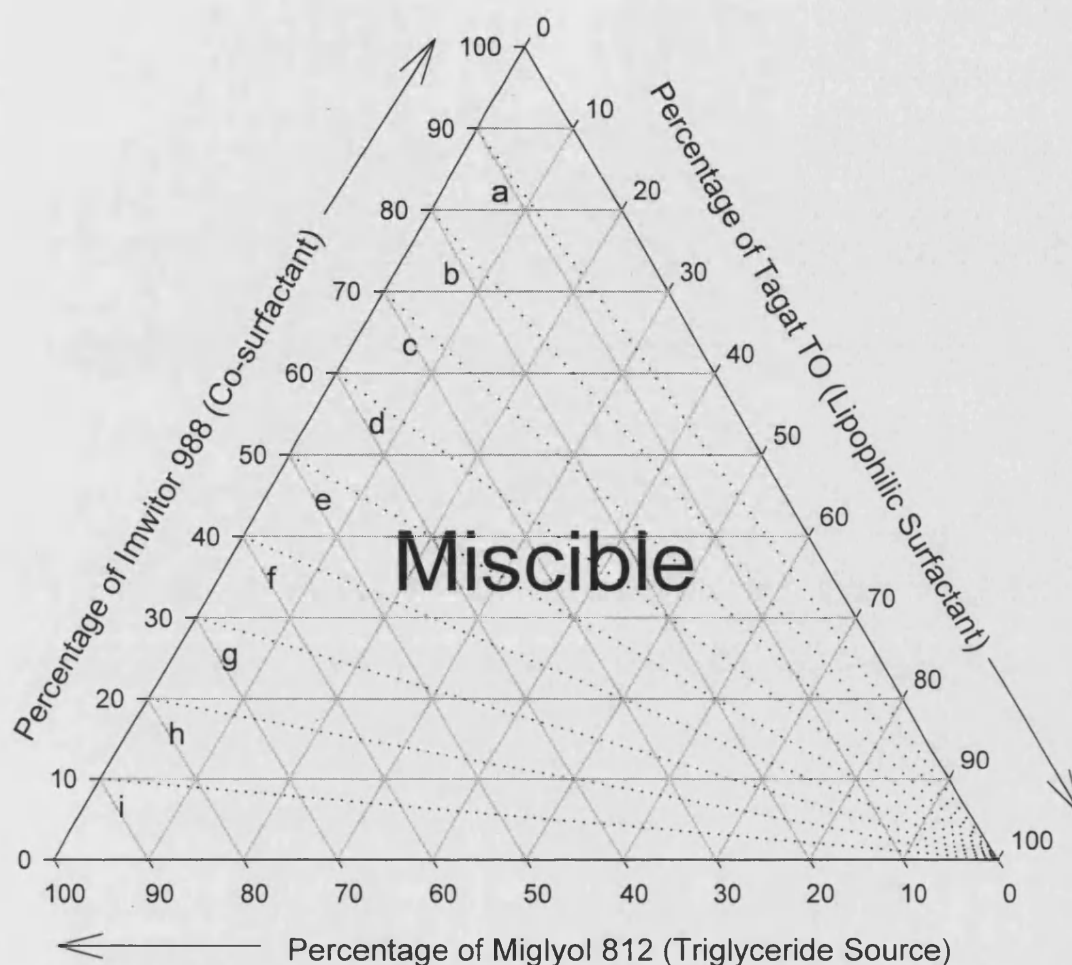
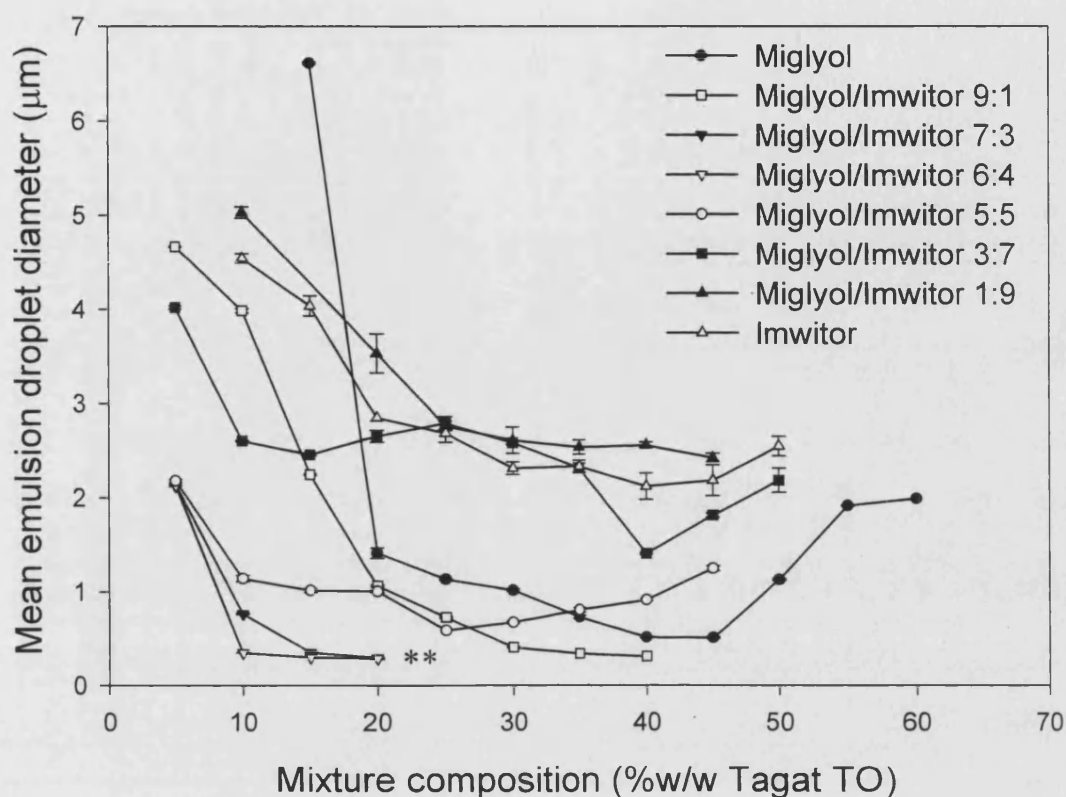


Figure 3.1.1

Ternary phase diagram for Miglyol 812/Imwitor 988-Tagat TO system displaying miscibility for formulations of various compositions. Dotted lines (A to I) represent increasing ratio fraction of Miglyol 812:Imwitor 988; 1:9, 2:8, 3:7 to 9:1, respectively. All points on each line have constant ratio of Miglyol 812:Imwitor 988 which represents that line diluted with increasing concentration of Tagat TO. The mean emulsion droplet diameter profiles for different Miglyol 812/Imwitor 988 ratios depicted in figure 3.2 correspond to these lines.



**Figure 3.1.2**

Mean emulsion droplet diameter (MEDD) profiles for self-emulsified systems containing different ratios of Miglyol 812/Imwitor 988 and increasing concentration of Tagat TO. Lipid formulations were emulsified in water at 37°C for 15 minutes. MEDD values were determined by laser diffraction method using the Mastersizer. Bars Represent Standard Errors ( $n = 3$ ). \*\*Size droplets of dispersions of oil systems containing Miglyol/Imwitor ratios of 7:3 or 6:4 at Tagat TO concentrations >20% could not analyzed using the Mastersizer due to the size range cut off of the apparatus.

concentration emulsions. Microscopical examination of these crude emulsions showed that oil droplets in excess of 500  $\mu\text{m}$  were present <sup>[8]</sup>. (2) Between 20 and 45% surfactant resulted in sub-micron emulsions of improved stability. The MEDD apparently reached a minimum value at surfactant concentration of 40-45%. The reduction in the MEDD in this second region probably resulted from increased interfacial stabilisation by water soluble surface active components as the surfactant concentration was increased. (3) At higher surfactant concentrations ( $> 50\%$ ) the MEDD increased with a doubling of the polydispersity. The turbidity of self-emulsified systems decreased as the surfactant concentration was increased from 45-60%. The increase in the MEDD values for optically clear systems containing more than 45% surfactant may be explained by the large swollen microemulsion systems described by Gerbacia and Rossano <sup>[10]</sup>, where the refractive indices of dispersed and continuous phases are very similar.

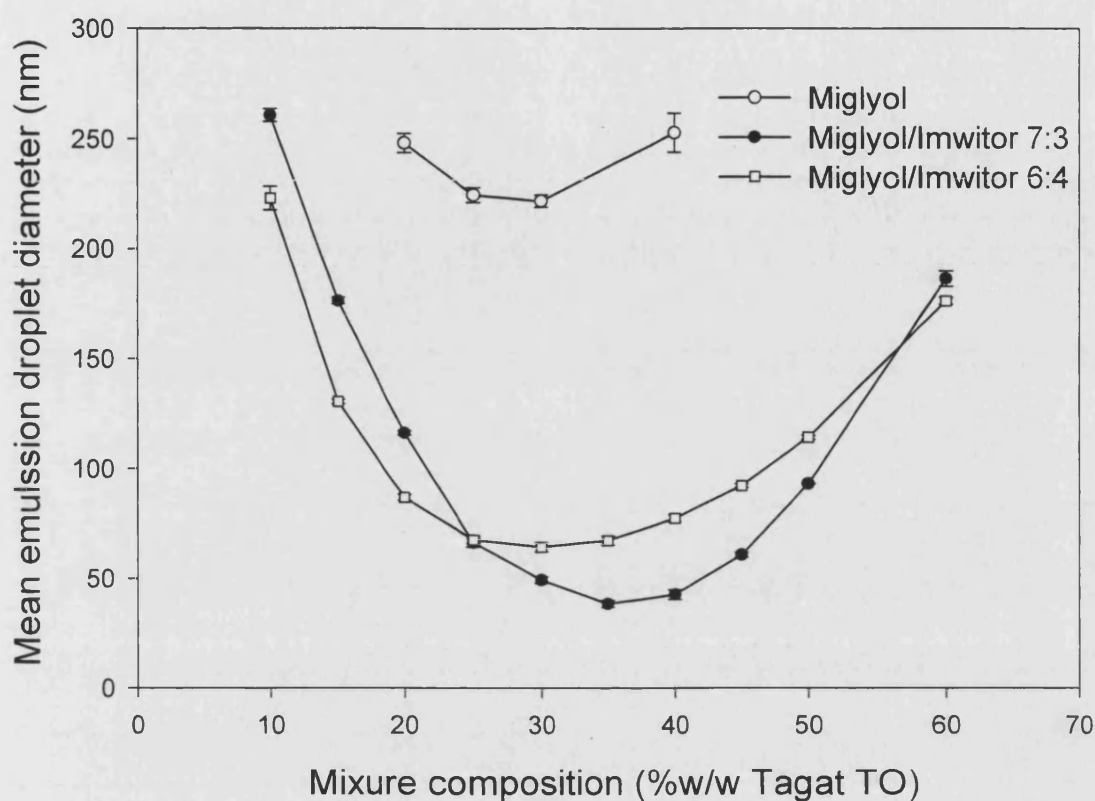
In comparison with the Miglyol 812-Tagat TO emulsification profile, the incorporation of Imwitor 988 at different ratios in the binary mixtures has produced three sets of self-emulsified systems (see figure 3.1.2). This dramatic effect on the emulsification behaviour has been dependent on the surfactant concentration and the Miglyol 812/Imwitor 988 ratio in the self-emulsifiable mixtures. (A) Less self-emulsifiable systems presented by an increase in the MEDD with reference to Miglyol 812/Tagat TO emulsification profile. This comprises the emulsification of Miglyol 812/Imwitor 1:9 and 3:7 ratios, and Imwitor 988/Tagat TO mixtures at surfactant concentrations above 15% w/w. The particle size-surfactant concentration profiles obtained for these formulations have shown two regions; at surfactant concentration between 5 and 25%, the increase in surfactant concentration accompanied by a corresponding decrease in the MEDD. Above 25% surfactant the MEDD stayed approximately unchanged regardless the increase in the surfactant, except for the ratio 3:7 where the optimum value occurred at 40% surfactant. (B) Improved self-emulsifiable formulations which include Miglyol 812/Imwitor 988 9:1 8:2 and 5:5 proportions. These formulations have produced particle size-surfactant concentration profiles comparable to the reference point (Miglyol 812/Tagat TO). However, MEDD values were relatively lower at all surfactant concentrations except for formulation 5:5 at Tagat TO concentration above 35% w/w, see table 3.1.1. Formulation 5:5 has

substantially reduced the MEDD at Tagat concentration as low as 5-15% in comparison to the crude or coarse dispersion produced by Miglyol 812-Tagat TO, 1:9 or 8:2 formulations. The MEDD apparently reached a minimum value at 30% surfactant concentration for 9:1 and 8:2 and at 25% for 5:5 formulations. This is far less than the optimum Tagat TO concentration required to obtain comparable minimum MEDD values in the case of Miglyol 812-Tagat TO system. (C) Microemulsion systems which include blends of Miglyol 812/Imwitor at 7:3 or 6:4 proportions. These systems have produced sub-micron emulsions at very low Tagat To concentrations (>10%) compared to the crude dispersion of Miglyol 812/Tagat TO at same concentrations. These formulations were at the lower detection limit of the Mastersizer at surfactant concentration above 20% as they formed typical bluish clear microemulsions. Therefore, these systems were further characterized using PCS technique.

**Table 3.1.1: Effect of Miglyol/Imwitor ratio on the self-emulsifying behaviour of Tagat TO-Miglyol 812 system as measured by mean emulsion droplet diameter using the Mastersizer.**

% Tagat TO (w/w)	Miglyol:Imwitor Ratio			Statistical	
	10:0	9:1	5:5	Difference p<0.05	
	MEDD ( $\mu\text{m}$ )			10:0/9:1	10:0/5:5
20	1.42 $\pm$ 0.06	1.07 $\pm$ 0.01	1.01 $\pm$ 0.02	✓	✓
25	1.14 $\pm$ 0.01	0.73 $\pm$ 0.01	0.59 $\pm$ 0.00	✓	✓
30	1.02 $\pm$ 0.01	0.41 $\pm$ 0.01	0.67 $\pm$ 0.01	✓	✓
35	0.73 $\pm$ 0.02	0.34 $\pm$ 0.00	0.81 $\pm$ 0.03	✓	No
40	0.51 $\pm$ 0.01	0.31 $\pm$ 0.00	0.91 $\pm$ 0.01	✓	✓

Results obtained from PCS for emulsions formed from Miglyol 812/Tagat TO and {Miglyol 812/Imwitor} (6:4, 7:3)-Tagat TO are shown in figure 3.1.3. Self-emulsifying mixtures of Miglyol 812/Tagat TO, produced a minimum MEDD at surfactant concentration between 25 and 30%. At surfactant concentrations between 20 and 40% for Miglyol 812-Tagat TO system, MEDD values measured by PCS



**Figure 3.1.3**

Mean emulsion droplet diameter profiles for Miglyol 812/Tagat TO and {Miglyol 812/Imwitor 988} (6:4, 7:3)-Tagat TO systems at increasing surfactant concentration. Oil formulations were emulsified in water at 37°C for 15 minutes. MEDD values were measured by photon correlation spectrometer (PCS). Bars represent standard errors ( $n = 3$ ).

method were lower than expected from the laser diffraction analysis using the Mastersizer, as depicted in figures 3.1.3 versus 3.1.2 respectively. This may have been due to underestimation by PCS of the MEDD of this system which probably contained droplets  $>3\mu\text{m}$ , which would have been excluded from any PCS analysis. Emulsions formed by mixtures containing less than 20% were poor and therefore, was not possible to detect any scattered light using the PCS at the normal sensitivity level i.e. the MEDD of such emulsions were always out of the range covered by the apparatus.

The particle size-surfactant concentration profiles as presented in figure 3.1.3 for {Miglyol 812/Imwitor} (7:3, 6:4), are of similar character exhibiting a minimum MEDD at about 30-40% Tagat TO. At Tagat TO concentration between 10 and 20% w/w, MEDD values for 6:4 system were relatively lower comparable to the 7:3 formulation at same surfactant concentrations, see table 3.1.2. However, at higher surfactant concentration (25-40%), 7:3 systems produced fine and almost clear dispersions at Tagat TO  $\geq 30\%$  with lower MEDD values comparable to 6:4. At surfactant concentrations  $> 45\%$  both systems 7:3 and 6:4 have produced though clear dispersions, yet, MEDD values have sharply increased with a doubling of the polydispersity. This could be attributed to the formation of swollen microemulsion systems.

**Table 3.1.2: Self-micro-emulsifying behaviour of 7:3 Formulations versus 6:4 with reference to Miglyol 812-Tagat TO system as measured by MEDD using QELS.**

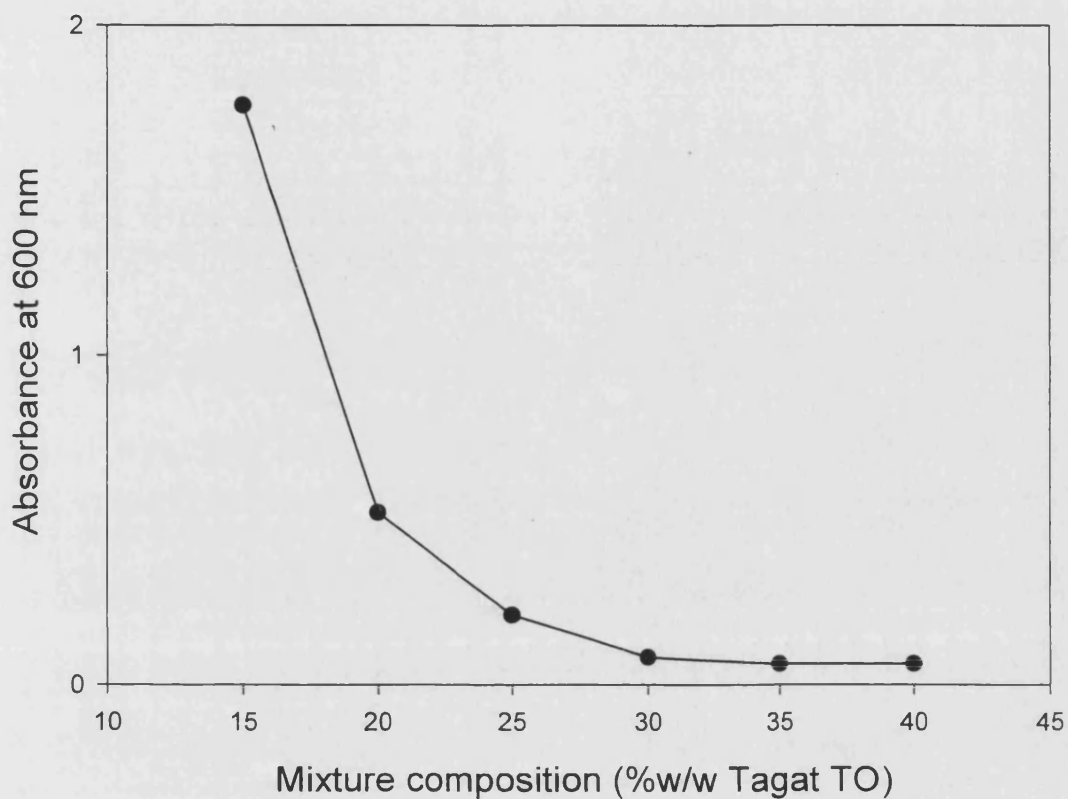
% Tagat TO (w/w)	Miglyol:Imwitor Ratio			Statistical Difference  p<0.05 7:3/ <u>6:4</u>
	10:0	7:3	6:4	
	MEDD (nm)			
10	off range	260.80±2.90	223.10±5.30	✓
15	off range	176.30±1.50	130.50±1.10	✓
20	248.10±4.38	116.30±1.00	87.00±1.30	✓
25	224.50±3.40	66.40±1.90	67.70±1.75	No
30	221.8±2.83	49.50±1.48	64.40±2.10	✓
35	256.15 2.05	38.60±1.60	67.40±2.00	✓
40	252.85 8.84	42.80±2.20	77.50±1.70	✓

In order to substantiate PCS sizing data for the microemulsion systems, turbidity was assessed by measuring the optical density of these emulsions with increasing surfactant concentration, see figure 3.1.4. A progressive drop in the UV absorbance corresponding to increases in the concentration of surfactant included in the oil formulation was observed as a result of particle size reduction. For Miglyol 812/Imwitor 988 at ratio of 7:3, almost optically clear dispersions with an absorbance at  $\lambda 600$  nm of 0.084 were obtained at optimum concentration of Tagat TO of 30% w/w. This correlates well with the particle size data presented for this system in figure 3.1.3 and table 3.1.2 whereby, optimum dispersions with MEDD values between 40 and 50nm and with polydispersity index around 0.061 (values between 0.1-0.15 represent highly mono-disperse systems) were produced.

In contrast to the crude dispersion of Miglyol 812/Tagat TO at surfactant concentration w/w 15% ( $>5\mu\text{m}$ ), emulsification of {Miglyol 812/Imwitor 988} 7:3 or 6:4 produced fine dispersion of  $130.5\pm 1.1$  nm and  $176.3\pm 1.5$  nm with polydispersity of  $0.065\pm 0.008$  and  $0.052\pm 0.023$ , respectively. Therefore, the inclusion of either three or four parts of Imwitor 988 in Miglyol 812/Tagat TO using only 15% w/w Tagat TO has produced a system which is comparing to Miglyol 812/Tagat TO (30%), has lower toxicity as less surfactant is used, and lower MEDD values with narrow size distribution. Moreover, it is expected to have better solubilizing capacity for poorly-water soluble drugs as Imwitor 988 is polar oil due to monoglyceride content.

Medium-chain ( $\text{C}_6\text{-C}_{12}$ ) fatty acids, mono-, di, and tri-glycerides, particularly  $\text{C}_8/\text{C}_{10}$  mono/diglycerides, have been used in mixed micelle and emulsion formulations as absorption enhancers of a number of different drugs <sup>[11]</sup>. In this investigation Imwitor 988 acts as a co-surfactant of similar effect of short chain alcohols in aiding the emulsification process of oil based formulations. The role of co-surfactant, such as a short chain alcohol, is to increase the interfacial fluidity by penetrating into the surfactant film and consequently creating a disordered film due to the void space among surfactant molecules <sup>[12]</sup>.

A crucial factor in forming a self emulsified system with fine droplets is the polarity of the resulting oil droplets which also promotes the fast release of the active ingredients into the aqueous phase. The polarity of oil is dependent on HLB, chain length and molecular weight of the hydrophilic part, and the surfactant concentration.



**Figure 3.1.4**

The effect of increasing Tagat TO concentration on the optical density for {Miglyol 812/Imwitor 988} (7:3)-Tagat TO system after dispersion in water as measured spectrophotometry. Oil formulations were emulsified in water at 37°C for 15 minutes. Absorbance at  $\lambda=600$  nm using the UV was measured to evaluate the turbidity of the resultant emulsions.



It is very well established amongst researchers the correlation between good emulsification and HLB of the oil mixture. However, this relation is highly affected by type and chemical structure of the surfactant; an issue which will be dealt with in forthcoming sections.

Theoretical HLB of the oil mixture can be calculated according the following equation:

$$HLB_{\text{mixture}} = f_A \times HLB_A + f_B \times HLB_B + f_C \times HLB_C$$

$F$  is the fraction of each excipient present in the formulation (A, B, or C).

**Table 3.1.3: Effect of HLB oil mixture on emulsification of Miglyol 812-Tagat TO system at 30% surfactant.**

Formulation composition	Miglyol	Miglyol:Imwitoror {7:3}	Miglyol:Imwitor {6:4}	Miglyol:Imwitor {5:5}
HLB <sub>mixture</sub>	4	4.84	5.12	5.40
Droplet size	221.80±2.83	49.5±1.48nm	64.4±2.1nm	215.60±3.90

As table 3.1.3 shows, the HLB margin for self-micro-emulsification in Miglyol 812 /Imwitor 988-Tagat system is very narrow, values between 4.84 and 5.12 are required. The inclusion of three to four parts of Imwitor 988 has produced an oil droplet with the optimum polarity and HLB required for micro-emulsification. It is important to emphasize that Imwitor 988 has equal proportions of mono and di-glycerides and considered to be polar oil due to monoglyceride content ( $HLB \approx 5$ ). Hence, oil polarity is enhanced by increasing the monoglyceride percentage as for example seen in Imwitor 308 which has 90% of mono-caprylate. The implication of using Imwitor 308 on emulsification behaviour in Miglyol 812 using various nonionic surfactants will be investigated from mechanistic perspective in sections to come.

### 3.1.1.2. Phase Behaviour: Mechanistics of Self-emulsification

Although self-emulsification is a dynamic non-equilibrium process involving interfacial phenomena, information about the self-emulsification can be obtained using investigations of equilibrium phase behaviour <sup>[13]</sup>. Emulsification efficiency of oil-surfactant systems (as determined from particle size) has been characterized and yet correlated with equilibrium phase diagrams.

Wakerly <sup>[6]</sup> profoundly studied binary mixtures of Miglyol 812-Tagat TO in an attempt to elucidate the mechanistic processes of emulsification and to highlight the important involvement of liquid crystalline phases forming at the interface in such processes, see section 1.6.1 in chapter 1. Therefore, the initial work was dedicated to studying the phase behavior of Miglyol-Tagat TO system as described by Wakerly. Hence, the effects of incorporating Imwitor 988 on the efficiency of emulsification of the binary mixtures will be established using equilibrium phase diagrams. Equilibrium phase behaviour was studied in two ways (1) Static composition dilution method and (2) Dynamic composition dilution method, described in sections 2.2.4.1 and 2.2.4.2, respectively.

In the phase diagrams, depicted in figures 3.1.5-10, aqueous-based liquids are denoted  $L_1$ , oil-based liquids  $L_2$ , and liquid crystalline phases  $LC$ . Multiphasic mixtures were clearly distinguished by their turbidity, and the presence of significant quantities of liquid crystalline material was established by strong birefringent patterns observed using the polarizing viewer. No attempt had been made to quantify the composition or volume fraction of the phases present in the multiphasic mixtures. Such studies are extremely tedious and were un-necessary for the purpose of this work. Turbid mixtures, which tended to separate on storage into a water-rich phase and an oil-rich phase, were designated  $(L_1 + L_2)$  or  $(L_1 + L_2 + LC)$  when  $LC$  material was clearly present. Two types of  $LC$  phases were identified: (1) The  $LC$  phase, was viscous and exhibited “white” birefringence (2) The  $LC_a$  phase, transparent liquid crystalline dispersion exhibited low viscosity and “multicoloured” birefringence, typical of lamellar liquid crystalline phases. The phase diagram notation that was used for all subsequent phase diagrams is based on Mitchell <sup>[14]</sup> and is summarised in table 3.1.4.

**Table 3.1.4: Summary of Phase Diagram Notation**

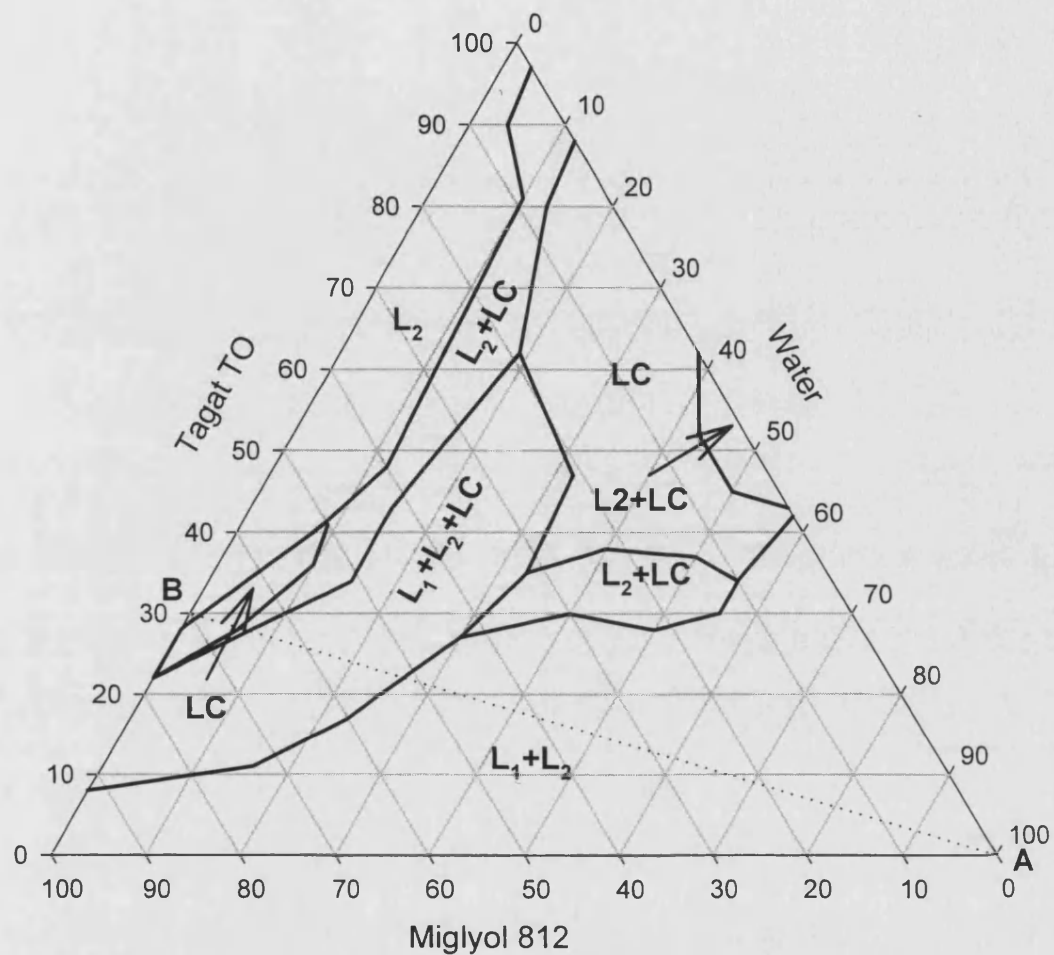
Phase Notation	Description of Phases
$L_2$	Isotropic oil continuous phase which contain dissolved water
$L_1$	Isotropic water continuous phase which contain dissolved oily component
$L_1+L_2$	Two phase system which can be either a stable emulsion, or separate aqueous s and oily continuous layers.
LC	Liquid crystalline phase
$LC_a$	Metastable liquid crystalline dispersion

#### *Equilibrium phase behaviour using static compositions*

The triangular equilibrium phase diagram for the Miglyol 812-Tagat TO-water system at 25°C is shown in figure 3.1.5. Phases were identified as isotropic ( $L_2$  or  $L_1$ ), liquid crystal (LC) or liquid crystal containing phase (e.g.  $L_2+LC$ ), or combination phase regions (e.g.  $L_1+L_2$ ). For simplicity no differentiation was made between the different liquid crystal phases in the initial equilibrium phase diagram studies.

In the self-emulsification experiments presented in figure 3.1.3, binary or ternary mixtures were diluted with water to give a final composition which was close to the water apex in the triangular phase diagram. It may be assumed that if stable emulsions are formed in these regions, good self-emulsification would be observed.

Considering the dilution of a 30% Tagat TO-Miglyol 812 mixture with water at 25°C (Figure 3.1.5), as depicted by the line A-B, the initial binary mixture ( $L_2$  phase) could pass through  $L_2 \rightarrow LC \rightarrow L_1+L_2+LC \rightarrow L_1+L_2$  phases. However, during self-emulsification of the binary mixture non-equilibrium multiple phases could have been formed. Therefore, the complete route depicted by line A-B may not be followed. Also, self-emulsification is a dynamic process hence, using equilibrium phase diagrams will only be relevant up to a point beyond which non-equilibrium phases



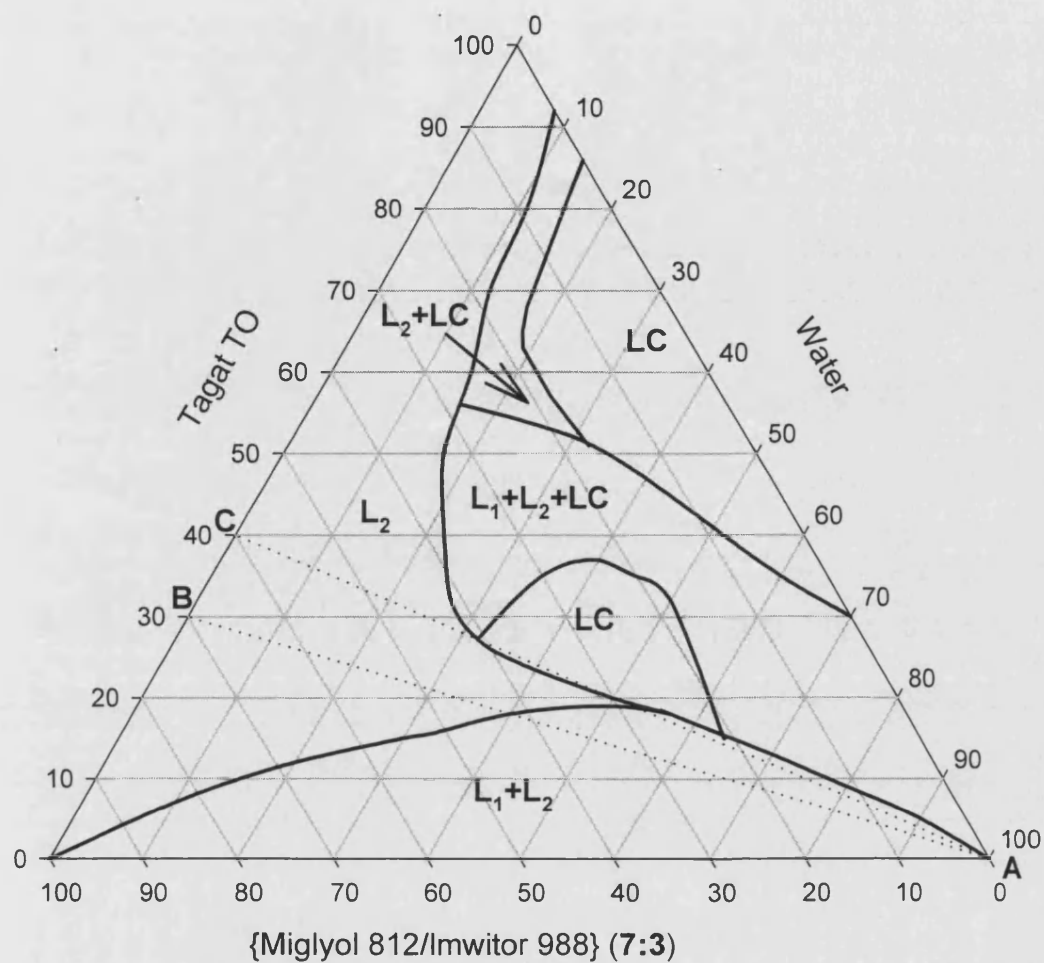
**Figure 3.1.5**

Triangular equilibrium phase diagram for the Miglyol 812-Tagat TO-water system at 25°C. Line A-B represents the dilution of Miglyol 812-Tagat TO oil mixture of ratio 70/30 with water. Aqueous-based liquids are designated  $L_1$ , oil-based liquids ( $L_2$ ) and liquid crystal phases “white” birefringence (LC).

exist. Therefore, Equilibrium phase behaviour using the dynamic composition dilution method (section 2.2.4.2) will be re-evaluated for all diagrams and presented in later sections. Construction of phase diagrams with respect to water content and temperature described by Shinoda and Friberg <sup>[15]</sup> enables detailed evaluation of the important areas of the diagrams in the region 0 to 40%

In his study Wakerly <sup>[6]</sup> by constructing the triangular equilibrium phase diagrams for the Tagat-Miglyol-water system at 25, 30 and 40°C concluded that, the presence of a  $L_1+L_2+LC$  region which only occurred to a great extent at 25°C is not essential for self-emulsification. It is the phases adjacent to the isotropic  $L_2$  region which probably control self-emulsification. Also, as different liquid crystal phases (LC) was not fully classified, it might have reflected in the difficulty of explaining the observed self-emulsifying behavior of Miglyol 812/Tagat TO system.

The triangular equilibrium phase diagram for {Miglyol 812/Imwitor 988} (7:3)-Tagat TO-water system at 25°C is shown in figure 3.1.6. The dilution with water of the {Miglyol 812/Imwitor 988} 70/30-Tagat TO system at ratio fractions of Tagat TO  $\leq$  40 (area between line A-C and below), produced initial ternary isotropic mixtures ( $L_2$ ) that could pass through  $L_2 \rightarrow L_1 + L_2$ . This region of the phase diagram, with respect to the Miglyol-Tagat TO-water system (Figure 3.1.5), has two significant features: (1) a huge enhancement in the  $L_2$  region and (2) no presence of liquid crystal phases. Maximum solubilization of water in the isotropic oil phase ( $L_2$ ) occurred at ratio fractions of {Miglyol 812/Imwitor 988} 70/30-Tagat TO between 70(70/30)30 and 60(70/30)40, as depicted by the lines A-B and A-C, respectively (Figure 3.1.6). The amount of water that could be contained in the oil mixtures at these ratios is 35-40% and 45-50% w/w, respectively. This indicates the fact that the enhancement in the  $L_2$  region is of paramount importance in the mechanistics of emulsification when Imwitor 988 is included in the system, especially as the region is clear from any presence of LC phases. This correlates well with the self-emulsification data for Miglyol 812/Imwitor 988 (7:3)-Tagat TO presented in figure 3.1.3. This system produced a minimum MEDD at surfactant concentration between 30 and 40%. The importance of  $L_2$  in the mechanistics of emulsification will be reflected upon when phase diagrams with respect to water content and temperature are discussed in sections to come. Nonetheless, at Tagat TO ratio fractions in the oil mixture of  $> 40$  (area between line A-C and above, figure 3.1.6), liquid crystal regions appeared, but



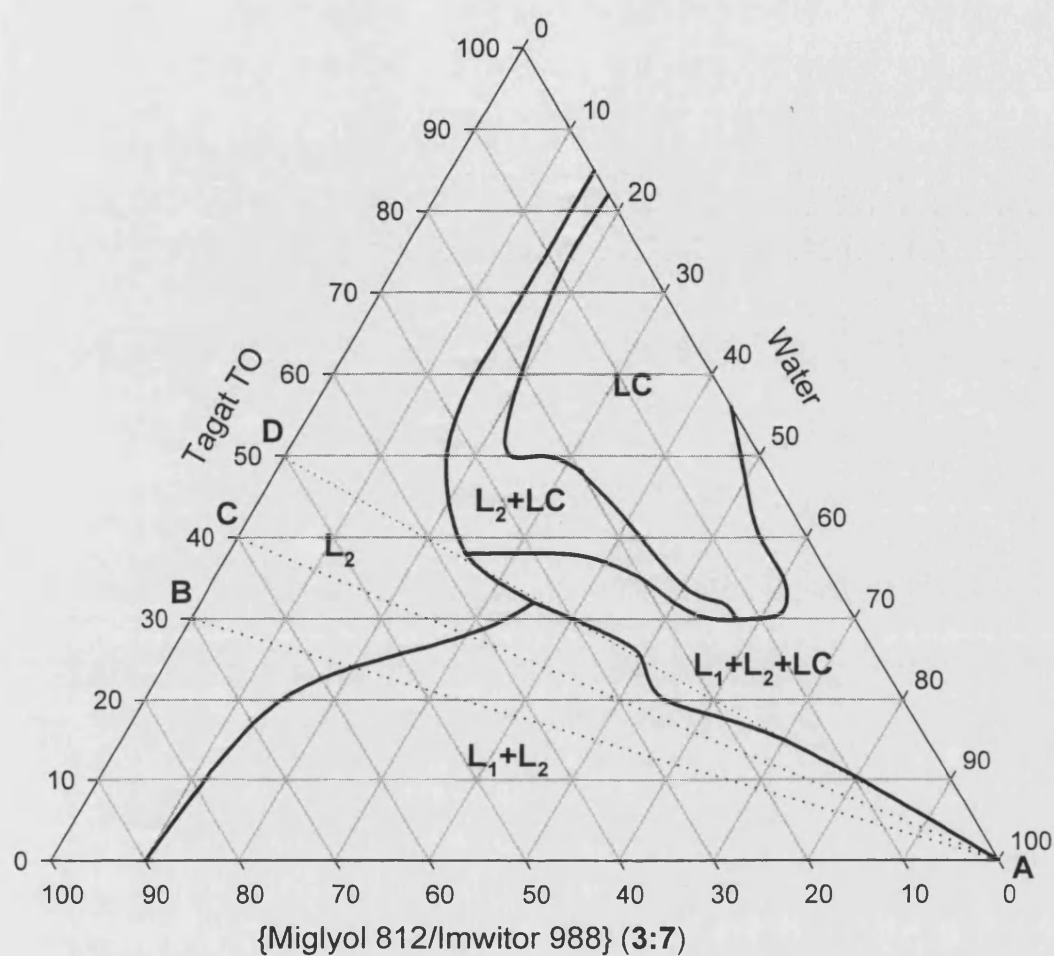
**Figure 3.1.6**

Triangular equilibrium phase diagram for the {Miglyol 812/Imwitor 988}(7:3)-Tagat TO-water system at 25°C. Lines A-B and A-C represent the dilution of oil mixtures of compositions [70(70/30)30] and [60(70/30)40] with water, respectively.

however, to the right of the phase diagram retrospective to the LC phases seen adjacent to the  $L_2$  region in the case of Miglyol-Tagat-water system, see Figure 3.1.5. The existence of LC regions at high Tagat TO concentration could be due to the ability of Imwitor 988 as polar oil to solubilise to a certain extent these liquid crystals at that particular temperature. This ability is up to a point beyond which Tagat TO becomes the dominant concentration in the system, and yet it is well known for nonionic surfactants to form LC phases on dilution with water.

The triangular equilibrium phase diagram at 25°C for {Miglyol 812/Imwitor 988} (3:7)-Tagat TO-water system is shown in figure 3.1.7. This system has shown a relative enhancement in the  $L_2$  region, vis-à-vis Miglyol-Tagat TO-water system (Figure 3.1.5). At Tagat TO ratio fractions in the oil mixture of 30 or 40 (lines A-B and A-C, Figure 3.1.7); the amount of water that could be solubilized in the oil mixtures is 15-20 and 25-30%, respectively. Yet, this increase in the  $L_2$  is relatively small in comparison with the enhancement observed at same Tagat TO ratios when only 3 parts of Imwitor was included (Figure 3.1.6). This might have reflected in the less self-emulsifiability of this system in comparison with Miglyol 812/Imwitor 988 (7:3)-Tagat TO, as depicted in figure 3.1.2. On the other hand, despite the enhancement in the  $L_2$  region in the case of Miglyol 812/Imwitor 988 (3:7)-Tagat TO retrospective to Miglyol/Tagat TO system, the latter system produced relatively less self-emulsifiable mixtures which reflects the importance of LC phases forming at the interface adjacent to the  $L_2$  phase when the expansion in the  $L_2$  is within certain limit. Maximum water solubilization of 30-35%w/w occurred at ratio fraction of Tagat TO of around 50, as depicted in the line A-D figure 3.1.7. This aqueous enhancement is comparable to the increase in the  $L_2$  region observed in the Miglyol/Imwitor (7:3) at Tagat TO ratio fraction of 30, see figure 3.1.6, yet, emulsification of Miglyol 812/Imwitor 988 (3:7)-Tagat TO system at ratios of 50(30/70)50 did not produce relatively fine dispersions. This could be due to the formation of large “swollen” microemulsion systems when relatively high surfactant proportions are used <sup>[10]</sup>.

Similar trend of phase behaviour was observed when no Miglyol 812 was used in the system. The triangular equilibrium phase diagram at 25°C for the Imwitor 988-Tagat TO-water system is shown in figure 3.1.8. At Imwitor-Tagat To ratios between 70/30 and 40/60 concentrations, maximum solubilization of water of 20-25%w/w resulted. This enhancement in the  $L_2$  region was not enough, compared to Miglyol/Imwitor (7:3)-Tagat TO (Figure 3.1.6), to produce good self-emulsification, as reflected in the



**Figure 3.1.7**

Triangular equilibrium phase diagram for the {Miglyol 812/ Imwitor 988}(3:7)-Tagat TO-water system at 25°C. Lines A-B, A-C and A-D represent the dilution of oil mixtures of compositions [70(30/70)30], [60(30/70)40] and [50(30/70)50] with water, respectively.



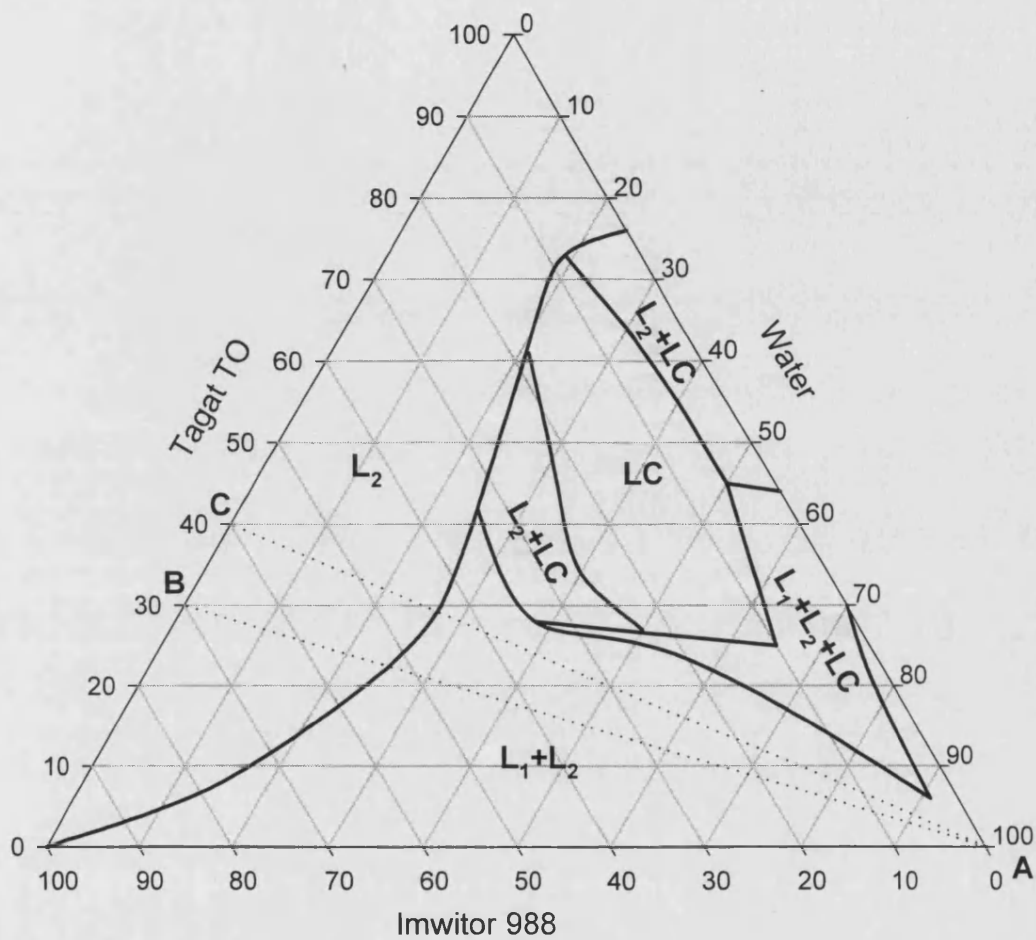


Figure 3.1.8

Triangular equilibrium phase diagram for the Imwitor 988-Tagat TO-water system at 25°C. Lines A-B and A-C represent the dilution of Imwitor 988-Tagat TO oil mixtures at ratio fractions of 70/30 and 60/40 with water, respectively.

particle size data presented in figure 3.1.2. This emphasises the importance of Miglyol 812 in aiding the emulsification process when Imwitor 988 is used in these systems.

### *Equilibrium phase behaviour using dynamic compositions*

Figures 3.1.9 and 3.1.10 depict the equilibrium phase behaviour for different mixtures of Miglyol 812/Imwitor 988-Tagat TO, on dilution with water. Comparative equilibrium phase studies are carried out on Miglyol-Tagat TO system with and without Imwitor 988 at Tagat TO concentrations 15 and 30%. This enables insight into the mechanistics of the observed changes in self-emulsification behaviour of these systems. Figure 3.1.9a displays the equilibrium phase diagram for a system containing 85 % (w/w) Miglyol 812 and 15%w/w Tagat TO diluted with water. The isotropic  $L_2$  region occurs below approximately 1% water at temperatures below 45°C. At temperatures between 45°C and 55°C water solubilization reached its maximum (2%). The dilution of the binary mixture by water (1 to 3.5%) resulted in a phase transition to  $L_2+LC_a$  at temperatures below 60°C. At higher water contents or at temperatures above 60°C Phase separation to  $L_1+L_2$  occurs. The presence of the small amount of  $LC_a$  in the  $L_2$  phase aided in the disruption of the oil continuous phase and thus slightly improved self-emulsification occurred <sup>[6]</sup>. Yet at 30% Tagat TO (Figure 3.1.10a) an extensive area of liquid crystal phase occurred which can be attributed to the optimum self-emulsification observed over the range 30 to 45% surfactant.

In his study Wakerly <sup>[6]</sup> has shown that the enhancement of the  $LC_a$  area corresponds to the increase of Tagat TO concentration. This enhancement is up to a point (50% surfactant) beyond which partial or total separation of  $LC_a$  into  $L_2$  and LC occurs and thus self-emulsification is impaired. He indicates also that the  $LC_a$  phase is in fact a dispersion of liquid crystals in isotropic  $L_2$  phase. At surfactant concentrations between 30 and 45% separation of  $LC_a$  into  $L_2$  and LC was rather difficult due to similarities in refractive indices and densities of the two components. Typically  $LC_a$  is optically clear and shows no tendency to separate even after storage for several months. However, ultra centrifugation at controlled temperature will enable partial separation of these dispersion phases.

The equilibrium phase diagram for a system containing Miglyol 812/Imwitor 988 (7:3) and 15% Tagat TO is shown in figure 3.1.9b. The inclusion of only 3 parts of

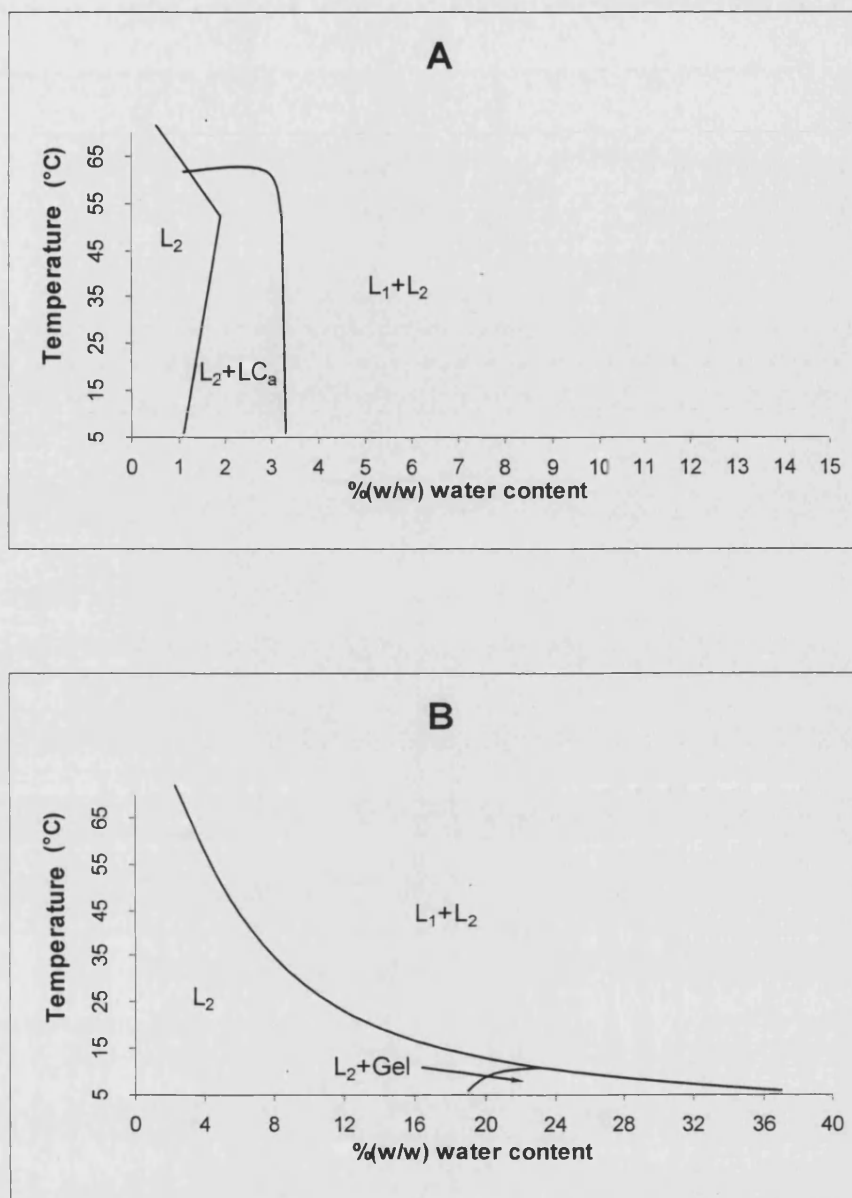


Figure 3.1.9

Equilibrium phase diagrams for Tagat TO-Miglyol 812 system at **15%** (w/w) surfactant on dilution with water (**A**) with no Imwitor 988 (**B**) with a Imwitor 988/Miglyol 812 ratio of 30/70. Aqueous-based liquids are designated  $L_1$ , oil-based liquids ( $L_2$ ); liquid crystal phases “white” birefringence (LC); and liquid crystal phases “multicolored” birefringence ( $LC_a$ ).

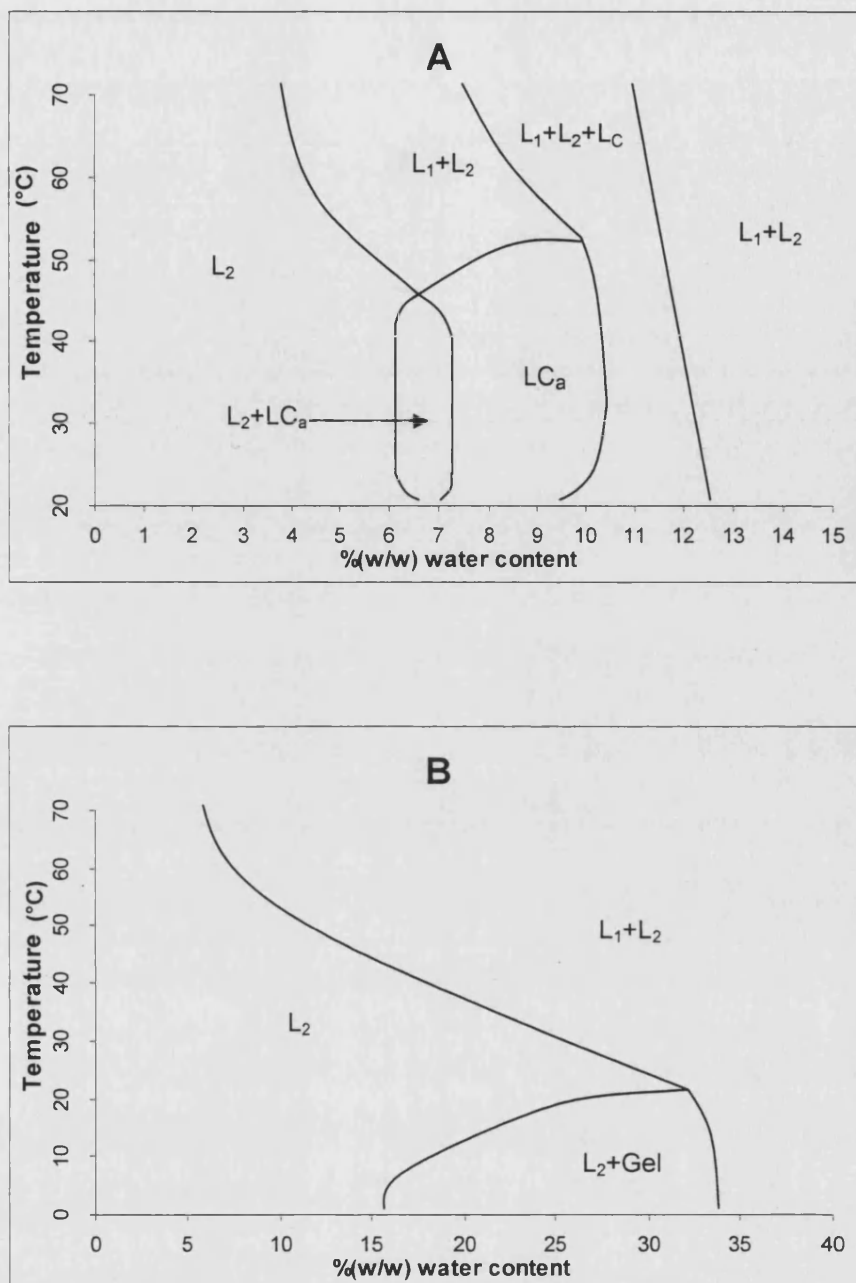


Figure 3.1.10

Equilibrium phase diagrams for Tagat TO-Miglyol 812 system at **30%** (w/w) surfactant on dilution with water (**A**) with no Imwitor 988 (**B**) with a Imwitor 988/Miglyol 812 ratio of 30/70.

Imwitor 988 has extended the area of  $L_2$  to almost 8% water at the emulsification temperature (37°C), with maximum solubilization of almost 22% water at 12°C. Whereas, in the previous system without Imwitor 988 (Figure 3.1.9a) the non-aqueous solubilization limit was approximately 2%. Improved emulsification observed in Miglyol 812/Imwitor 988 (7:3)-Tagat TO (15%) system was attributed to the enhancement in the  $L_2$  region (see table 3.1.5).

In the system containing 70 %(w/w) Miglyol 812 and 30%w/w Tagat TO (Figure 3.1.10a), maximum water solubilization ( $L_2$ ) extended to 7% at 45°C. Moreover, large area of liquid crystal phase ( $LC_a$  and  $L_2+LC_a$ ) occurred on further dilution with water by 6 to 10%w/w at temperatures below 50. On the other hand, the effect on phase behaviour of incorporating 3 parts of Imwitor in the system containing Miglyol 812 and 30% Tagat TO is shown in figure 3.1.10b. Substantial extension to the  $L_2$  region was observed reaching a maximum of almost 32-33 % water at 20°C. Water solubilisation at 37°C (emulsification temperature) was approximately 22-23%. This means that there is almost 4-fold enhancement in the  $L_2$  region at 37°C when 3 parts of Imwitor 988 was included in the Miglyol 812-Tagat TO (70/30) system. This has reflected in the fine dispersion obtained from the emulsification data of that system, see table 3.1.5.

As depicted in these phase studies, there are two distinctive features that may correlate to the efficiency of emulsification; (a) a region of enhanced water solubilization typical of non-ionic surfactant systems and thought to be a phase inversion region (b) the formation of a lamellar liquid crystalline dispersion phase on further incorporation of water. The occurrence of such stable or metastable liquid crystal dispersions at the oil-water interface, based on loosely associated aggregation structures was suggested to allow rapid penetration of water and disruption of the interface <sup>[16]</sup>. The resulting disruption will promote rapid self-emulsification.

The enhancement in the water solubilisation region ( $L_2$ ) becomes primarily the putative mechanism of self-emulsification when oil systems do not form any liquid crystalline dispersion. The importance of the  $L_2$  region in the mechanistics of emulsification will be further clarified when the changes on the phase behaviour induced by Imwitor 988 are discussed below.

The inclusion of Imwitor 988 in the binary mixtures (Miglyol-Tagat TO) has substantially transformed the phase behavior of these oil systems. It has induced three major changes in the phase diagram structure of the binary mixtures:

*(1) Changes in  $L_2$  regions*

An extensive enhancement in the  $L_2$  region reaching up to 35-40% water in some systems is observed. The inclusion of Imwitor 988 adds to the total polarity of the oil droplets as it is classified as polar oil due to the high content of monoglyceride ( $\approx 50\%$ ). This renders the propensity of the oil system to solubilizing more water up to a point beyond which phase transition to  $L_1+L_2$  occurs. Initially, aqueous penetration into the oily phase results in hydration of the oxyethylene part of the surfactant and also other polar components in the formulation. When the limit of the non-aqueous solubilization is reached phase separation to  $L_1+L_2$  occurs. It is likely that further dilution of these dispersions ( $L_1+L_2$ ) results in crude emulsions in systems such as Miglyol 812 at low Tagat TO concentrations 5, 10 and 15% whereby,  $L_2$  phase does not exceed 2%. Yet, in some oil systems such as Miglyol 812/Imwitor 988 (7:3)-30% Tagat TO whereby  $L_2$  region extends to  $\approx 35\%$  water at  $25^\circ\text{C}$ , further dilution of  $L_1+L_2$  dispersions transforms the system into micellar solution ( $L_1$ ).

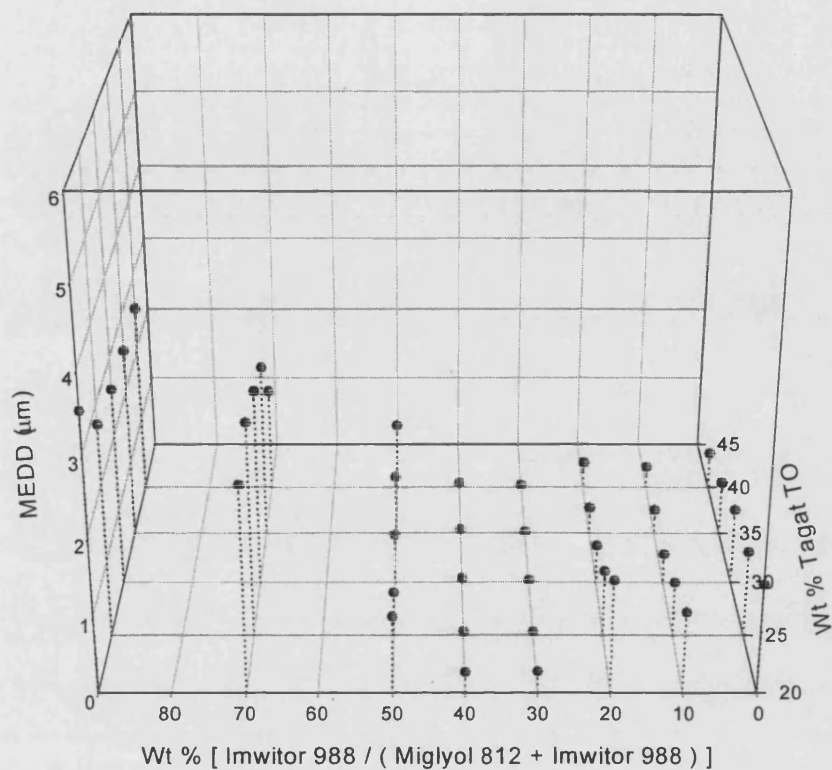
Therefore,  $L_2$  phase is crucial in the mechanistics of self-emulsification and thus the particle size distribution of the emulsified systems. It is rather important to address the properties of the  $L_2$  phase on the molecular level to enable insight into the self-emulsification process. According to criteria for the identification of microemulsions,  $L_2$  phase in aqueous systems of polar lipids and triglyceride oil is w/o microemulsion. On the basis of an X-ray study obtained by cooling  $L_2$  phases<sup>[17]</sup>, it was proposed that the structure consists of water lamellae separated by lipid bilayers corresponding to liquid-crystalline phase (see Figure 1.10c). It is important to note here that the increase in water content results in increased molecular disorder within the lipid molecule towards the hydrocarbon tail, this disorder corresponds to the swelling of the lamellar phase ( $L_2$ ). Therefore, the ability of these systems to solubilise water is due to the swelling behaviour of the  $L_2$  phase.

Based on a study by Rang and Millar <sup>[18, 19]</sup> investigating the emulsification of oils containing hydrocarbon, nonionic surfactant and *n*-octanol, the following mechanism for the self-emulsification of Miglyol 812-Imwitor 988-Tagat TO system can be proposed. Addition of the oil mixture to water results in the formation of interface between the oil and aqueous-continuous phases. Solubilization of water with the oily phase results from aqueous penetration through the interface. This will occur until maximum solubilization ( $L_2$ ) is reached close to the interface. Further aqueous penetration results in diffusion of the polar component of Imwitor 988 (1-Monocaprylate) and also to some extent Tagat TO into water. Subsequently, the system becomes supersaturated in oil and thus spontaneous nucleation of small oil droplets from the microemulsion occurs (i.e. phase transition from  $L_2$  to  $L_1+L_2$ ). Further diffusion of Tagat TO and monocaprylate out of the  $L_2$  phase forms a system that is ever more hydrophilic so that it eventually becomes miscible with water. The particle size of the nucleated oil droplets formed due to the diffusion process is expected to be related mainly to the interfacial and rheological properties of the  $L_2$  phase; the larger the ( $L_2$ ) area in the phase diagram the smaller the size of the nucleated oil droplets and vice versa.

Optimal range of initial drop compositions is required to obtain an emulsion of small oil droplets. According to our findings spontaneous micro-emulsification yielding uniformly fine dispersions was achieved when the injected drop was slightly lipophilic; i.e. the ratio of Imwitor 988-to-Miglyol 812 was slightly greater than that of the excess oil phase in equilibrium with a bicontinuous microemulsion at the experimental temperature, and when sufficient Tagat TO was used. As depicted in figures 3.1.11 and 3.1.12, smaller MEDD values were seen for oil formulations with Imwitor 988/Miglyol 812 ratios of 30/70 and 40/60. The need for this slightly lipophilic oil composition is attributed to the relatively low HLB value of Tagat TO (HLB $\approx$ 11). However, in the case of surfactants with higher HLB values, less lipophilic oil composition is required, as will be presented in section 3.2.

## (2) *Changes in the Phase Boundary Temperatures (PBT)*

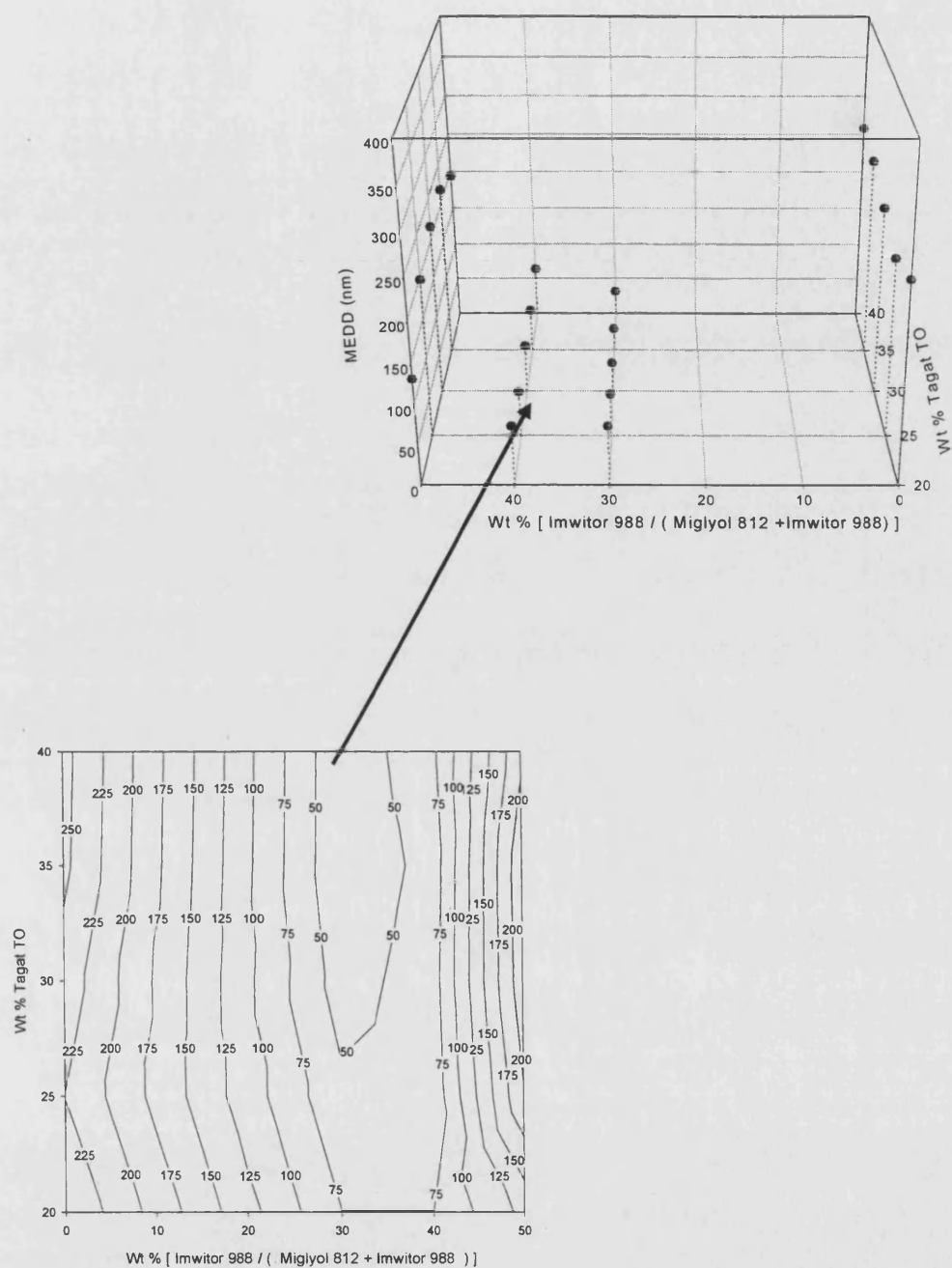
The phase inversion temperature (PIT) as defined by Friberg *et al* <sup>[20]</sup> is the temperature at which the emulsifier shifts its preferential solubility from water to oil



**Figure 3.1.11**

Three-dimensional graph of the effects of surfactant concentration and ratio of Imwitor 988/Miglyol 812 in the oil blend on the self-emulsifying performance of Miglyol 812/Imwitor 988-Tagat TO system as measured by mean emulsion droplet diameter using the Mastersizer.





**Figure 3.1.12**

Three-dimensional graph and contour plot representations of MEDD data for the emulsification of Miglyol 812/Imwitor 988-Tagat TO system as a function of surfactant concentration and ratio of Imwitor 988/Miglyol 812 in the oil blend. The highlighted area represents the optimum blend of Miglyol 812 and Imwitor 988 which produced the finest dispersions. MEDD values were determined using QELS.

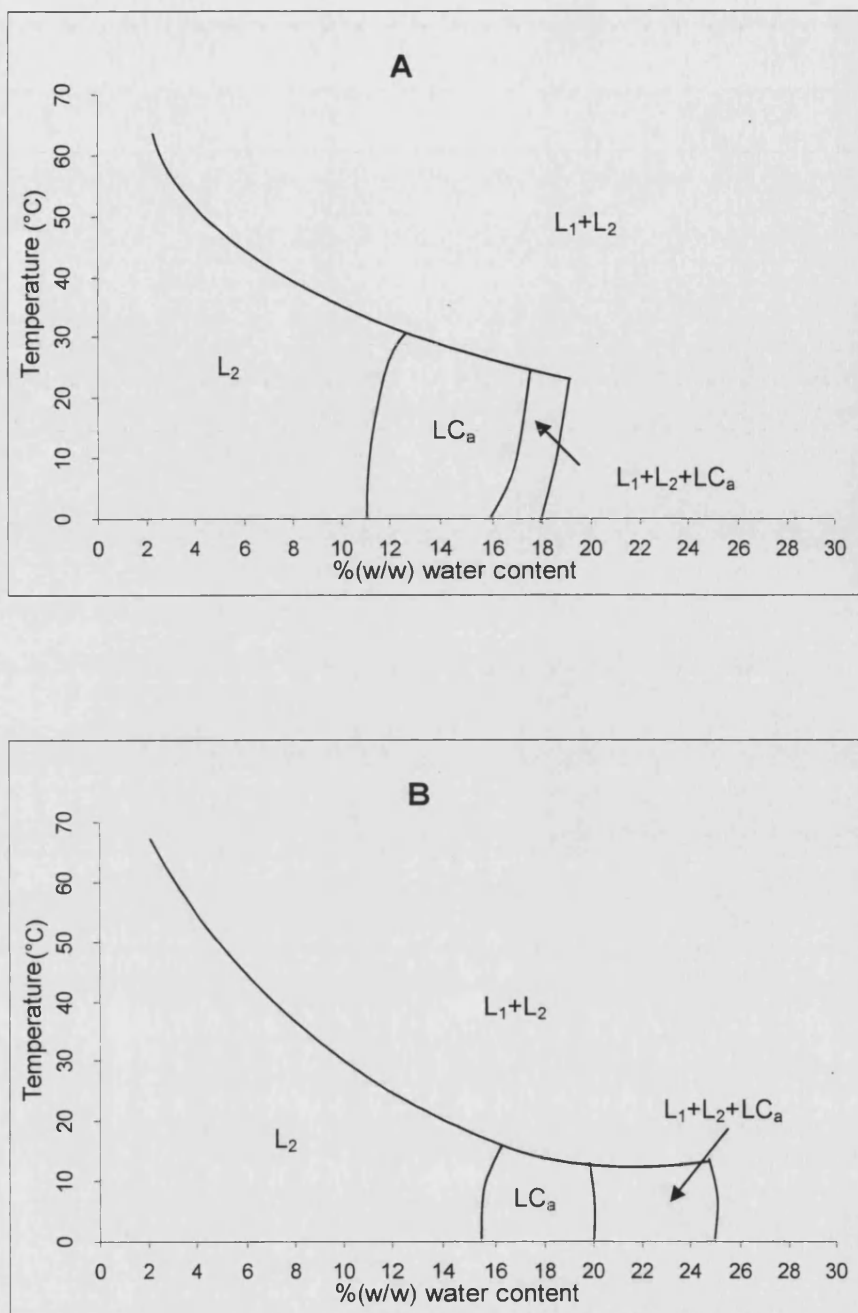
at elevated temperatures. The prime intension of this phase study has not been specifically directed to determine the PIT of these systems as this has to be investigated inextricable from droplet size analysis which will be addressed when we discuss the effect of emulsifying temperature. More importantly, however, equilibrium phase studies have been used to investigate the isotropic oil continuous phase ( $L_2$ ) and the lamellar liquid crystalline phase (LC), and how they interplay in the mechanistic processes. The minimum temperature at which phase transition from  $L_2$  to LC and  $L_1+L_2$  occurs is referred to here as phase boundary temperature (PBT) and has a significant role which underlines the PIT of the system. This temperature (PBT) apparently is not equivalent to the PIT whereby phase inversion from Winsor I to Winsor II takes place somewhere in the  $L_1+L_2$  region. Nonetheless, any depression in the PBT due to for example, increase in the temperature of the system, inclusion of polar oil, or addition of electrolytes reflects monotonically on the PIT of the system.

The equilibrium phase diagram for Miglyol 812-Tagat TO depicted in figure 3.1.10a displays PBT at around 45°C. Yet, it is evident from figures 3.1.13(a, b) that the inclusion of 1 part or 2 parts of Imwitor 988 in the system depresses PBT to 30°C and to 15°C, respectively. This could be attributed to the fact that Imwitor 988 as polar oil increases the solubility of the surfactant in the oil phase thus less temperature is needed to exert phase inversion. Therefore, it is expected from Imwitor 988 to reduce the PIT of the oil system when it is included in the Miglyol 812-Tagat TO mixtures.

### (3) *The Formation of Gel Phase*

As figures 3.1.9b and 3.1.10b show, the inclusion of 3 parts of Imwitor 988 in the Miglyol 812-Tagat TO system results in the formation of gel phase at temperatures below 15°C for the first system and below 20°C for the second. The gel phase normally consists of crystalline lipid bilayers alternating with water layer. When the  $L_\alpha$  phase is cooled below the temperature at which the hydrocarbon chains crystallize, a gel phase can be formed which is metastable.

As it has been discussed earlier,  $L_2$  phase is regarded as a lipid bilayer type of structure corresponding to lamellar liquid-crystalline phase  $L_\alpha$ . Therefore, the gel



**Figure 3.1.13**

Equilibrium phase diagrams for {Miglyol 812/Imwitor 988}-Tagat TO system at **30%** (w/w) surfactant on dilution with water **(A)** {Miglyol 812/Imwitor 988} ratio of 90/10 **(B)** Miglyol 812/Imwitor 988 ratio of 80/20.

phase which was seen in figures 3.1.9a and 3.1.10b could be due to the cooling of L<sub>2</sub> phase below the hydrocarbon-chain crystallization temperature. Moreover, it might be attributed to the monoglyceride content in Imwitor 988 viz monoglycerides in water form mesophases which by cooling can form gel phases.

**Table 3.1.5: Summary of the effects of Miglyol 812/Imwitor 988 blends on the phase behaviour description of {Miglyol 812/Imwitor 988}-Tagat TO system.**

Miglyol 812 at Tagat TO (15%)			Miglyol 812 at Tagat TO (30%)			
No of parts of Imwitor 988		Description	No of parts of Imwitor 988			
0	3	Of phase Behaviour	0	1	2	3
2%	22%	L <sub>2</sub> max.	7%	12%	16%	32
+	-	LCa	+++	++	+	-
-	+	Gel phase	-	-	-	+
60	15	PBT (°C)	45	30	15	20
coarse dispersion	176.30	Particle size (nm)	221.80	202.40	173.40	49.5

### 3.1.1.3. The Effect of Emulsifying Temperature and Medium on the Self-emulsification of Miglyol 812/Imwitor 988-Tagat TO Mixtures.

#### *Temperature variation*

It is important from stability prospective in emulsions to investigate the optimum temperature for stable emulsifications, the optimum hydrophilic chain length and the phase inversion temperature (PIT) of emulsifiers. Non-ionic surfactants often become increasingly more lipophilic at elevated temperatures. The change in micellar shape, size and eventual loss of aqueous solubility with increase in temperature is due to decreased hydration of the surfactant. Hydrogen bonding forces, which account for

aqueous solubility, are reduced at elevated temperatures. This is analogous to reducing the HLB of the surfactant.

In a similar manner an alteration of the hydrophilic chain length of the emulsifier should have an effect similar to the temperature change of the system since the interaction between the hydrophilic moiety and water is modified with variation in temperature <sup>[15]</sup>.

The change in nonionic surfactant-oil-water system (nSOW) phase behaviour with temperature can result in the phase transitions Winsor's type I to type III to type II (see figure 1.2b in chapter 1). Therefore, the higher the emulsification temperature is than the PIT of the emulsifier, the larger the emulsion droplets will be. Emulsification at temperatures slightly below the PIT (about 2-4°C below) affords optimum oil droplets of an o/w-emulsion type. On the other hand, emulsions are most unstable against coalescence at the PIT, due to the ultra-low interfacial tension <sup>[21]</sup>.

The effect of the emulsification temperature on the mean volume diameter of o/w-type emulsions of Miglyol 812-Tagat TO system with or without Imwitor 988 is shown in figures 3.1.14 to 3.1.16. For the system containing 85% Miglyol 812 and 15% Tagat TO as figure 3.1.14 illustrates, at emulsification temperature 35°C minimum oil droplets was obtained. A sharp increase in the mean emulsion diameter (from  $4.22 \pm 0.05$  to  $8.16 \pm 0.32$   $\mu\text{m}$ ) occurred at emulsification temperatures above 40°C. This suggests that phase inversion to w/o-type emulsion due the loss of the surfactant's affinity for the aqueous phase has occurred which resulted in larger emulsion droplets.

Nonetheless, optimum temperature for emulsification occurred at 20°C when the system above contained 3 or 4 parts of Imwitor 988 (7:3, 6:4), and at 30°C when 5 parts of Imwitor was used (Figure 3.1.15). There is obvious increase in the MEDD values concomitants the emulsification at increasing temperatures which reflects a sort of phase inversion behaviour. Nonetheless, due to the presence of Imwitor 988 which acts as a cosurfactant in enhancing the emulsification process, there has been no such formidable increase in the MEDD observed in the system without Imwitor 988 (Figure 3.1.14).

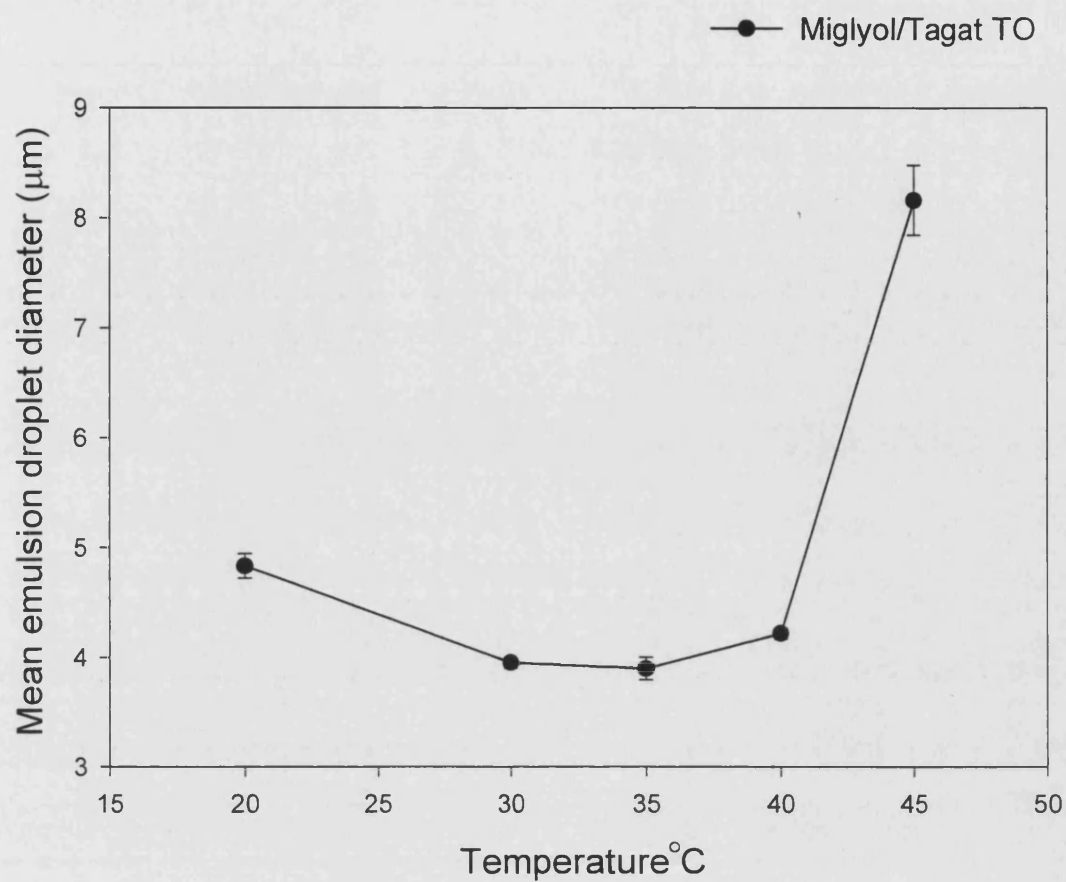


Figure3.1.14

The effect of self-emulsifying temperature on the mean emulsion droplet diameter for the Tagat TO-Miglyol 812 system at 15% (w/w) surfactant as determined by the Mastersizer. Bars represent standard errors (n = 3).

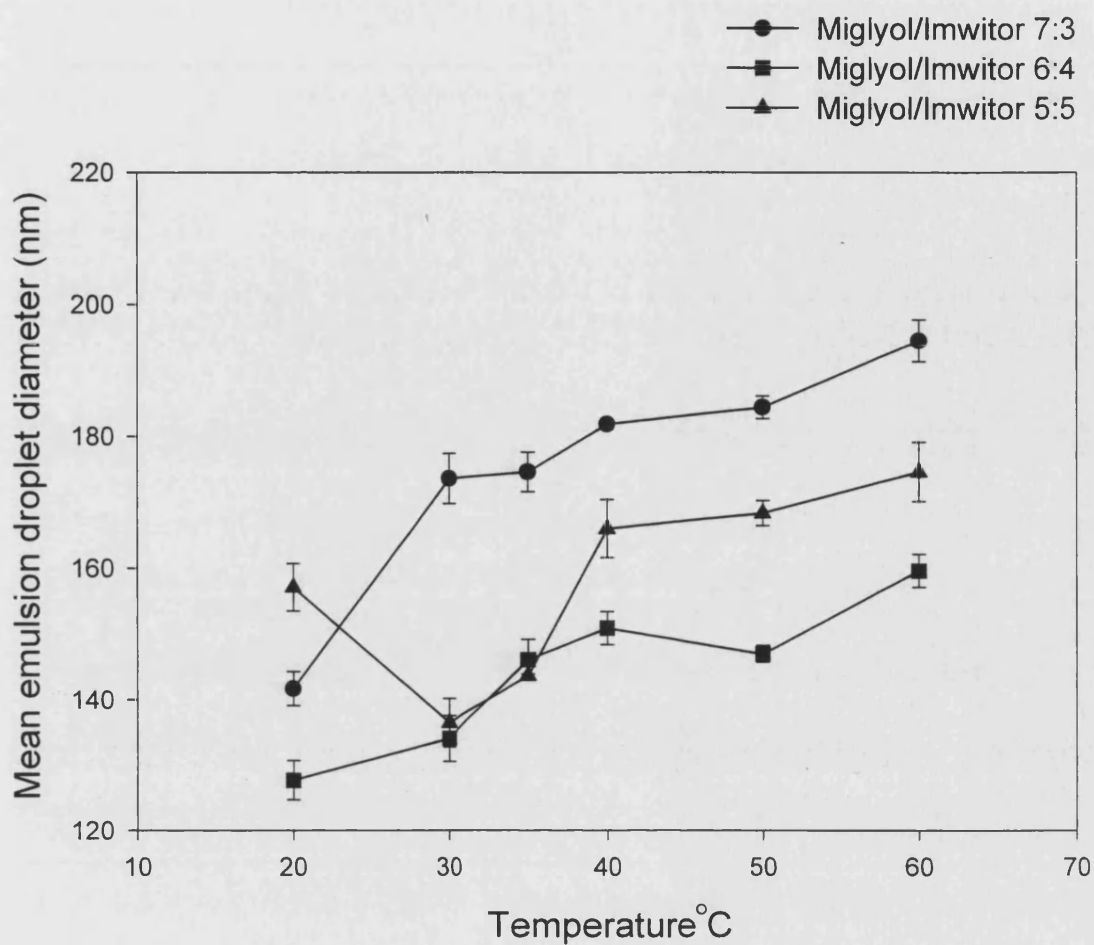


Figure 3.1.15

The effect of self-emulsifying temperature on the self-emulsifying performance of different ratios of Miglyol 812/Imwitor 988 at 15% (w/w) Tagat TO as measured by mean emulsion droplet diameter using QELS. Bars represent standard errors ( $n = 3$ ).

Figure 3.1.16 illustrates the effect of temperature on the MEDD of Miglyol 812/Tagat TO, and {Miglyol 812/Imwitor 988} (7:3), (6:4) and (5:5) using 30% w/w Tagat TO. These mixtures exhibit good self-emulsifying behaviour in terms of producing fine dispersions. Optimum MEDD values were obtained for Miglyol 812/Tagat TO and Miglyol 812/Imwitor 988 (5:5) using 30% Tagat TO at emulsification temperature 30°C. For Miglyol 812/Imwitor (7:3) and (6:4) using 30% w/w Tagat TO, almost clear microemulsion dispersions of minimum particle size were produced at temperatures between 35 and 40°C.

Quantitatively, oil systems depicted in figure 3.1.16 had shown no large increase in the MEDD at high emulsification temperatures comparable to the particle size increase observed when these systems contained 15% surfactant (figures 3.1.14 and 3.1.15). Qualitatively, however, clear dispersions obtained by the emulsification of Miglyol 812/Imwitor 988 (7:3) or (6:4) using 30% w/w Tagat TO turned turbid at temperatures above 45°C, yet on cooling to the room temperature for size measurement they became clear again. This phenomenon has been observed by many researchers and considered to be one of the distinctive criteria for microemulsions.

Formulations presented in figure 3.1.16 are considered robust oil systems in terms of self-emulsifying behaviour. Moreover, due to relatively high surfactant concentration (30% w/w), any reduction in the surfactant's aqueous solubility due to temperature variations will not result in significant increase in the MEDD as there will be enough surfactant to aid in the emulsification.

These studies show that in terms of minimum particle size and therefore potential ability to penetrate between the micro-villi of the gastrointestinal mucosa thus presenting drug to the absorption site, including 3 or 4 parts of Imwitor 988 in the system would be preferable when Tagat TO is used at concentrations 30 or 15%, respectively.

#### *Emulsification Media*

Since the luminal environment in the proximal GI tract varies considerably with site and meal ingestion, it is essential to consider the use of several different sets of



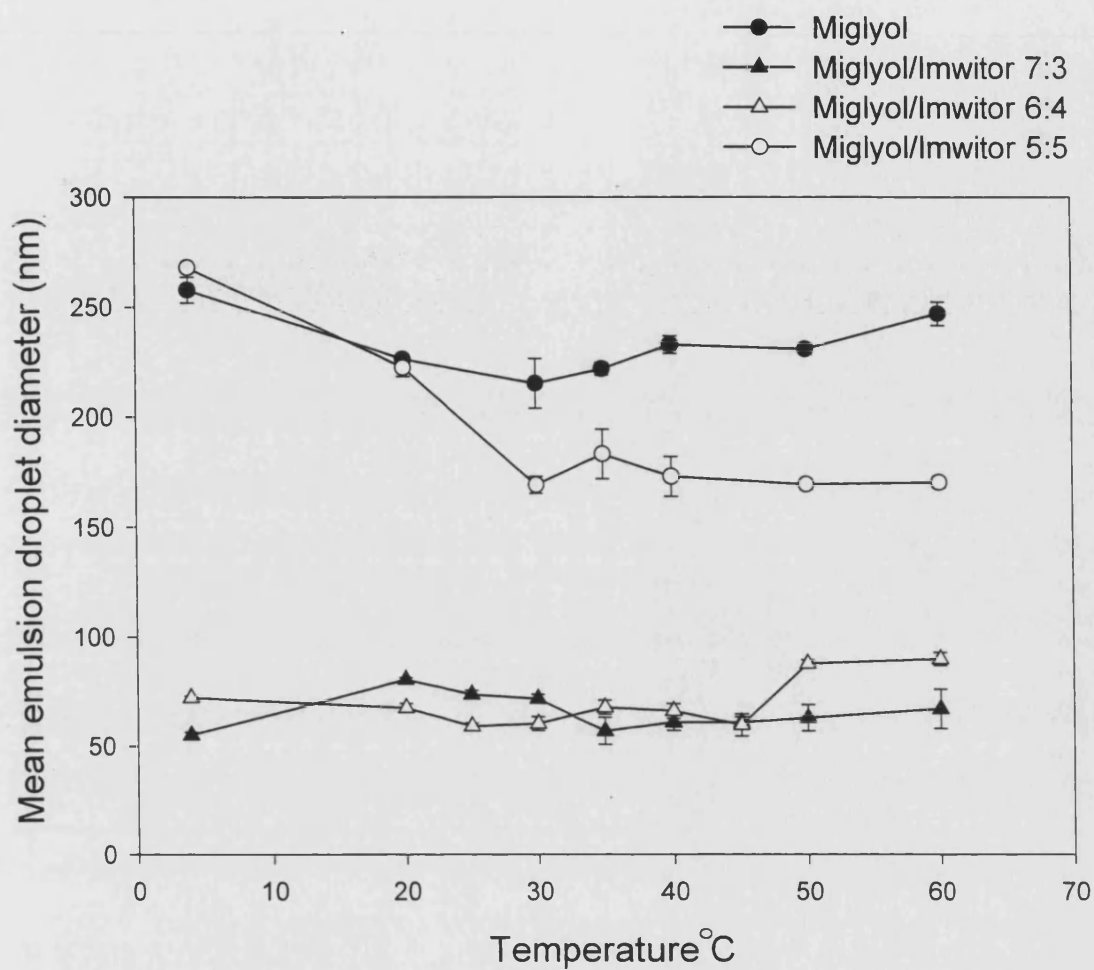


Figure 3.1.16

The effect of self-emulsifying temperature on the self-emulsifying performance of different ratios of Miglyol 812/Imwitor 988 at **30%** (w/w) Tagat TO as measured by mean emulsion droplet diameter using QELS. Bars represent standard errors (n = 3).

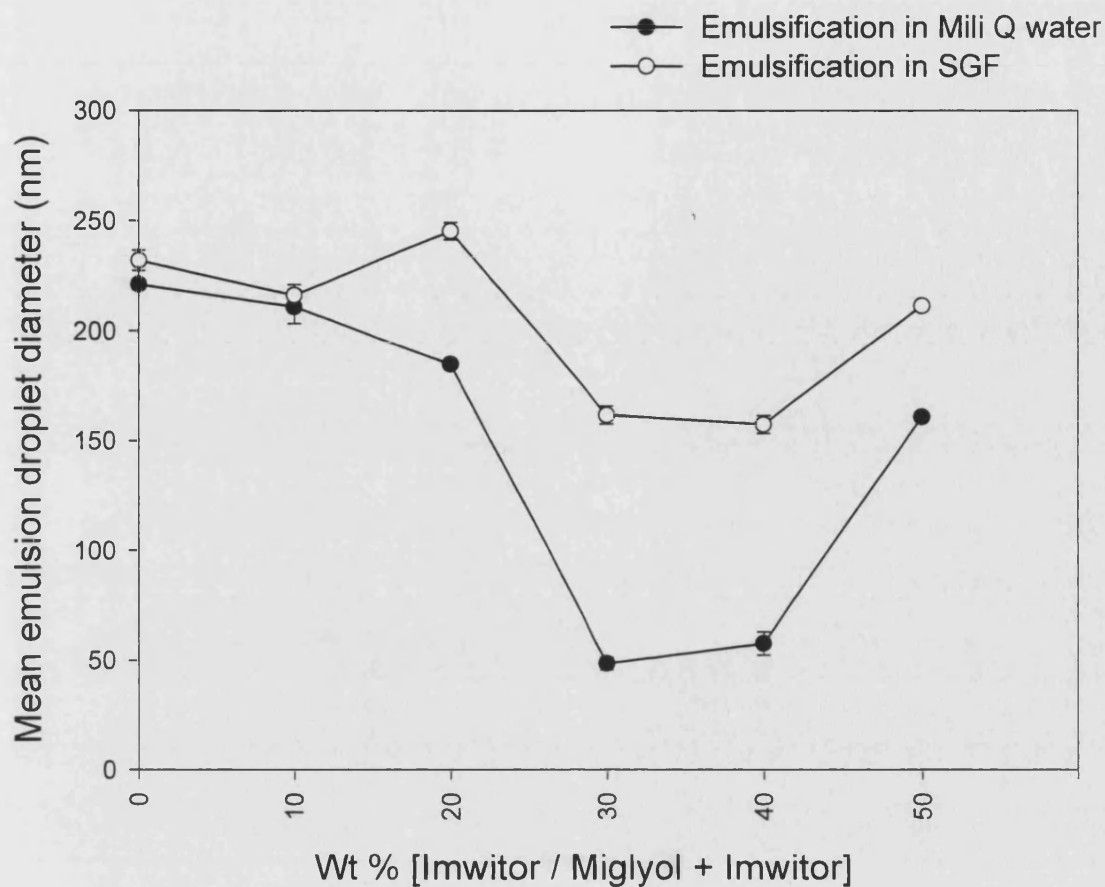
emulsification conditions to assess the dissolution behaviour of the oil formulations with and without drugs.

The United States Pharmacopoeia <sup>[22]</sup> calls for the Simulated Gastric Fluid (SGF) to simulate dissolution in the stomach. SGF simulates pH conditions in the fasted stomach. In the fed state, however, composition in the stomach will be highly dependent on the ingested meal. Long-life milk <sup>[23]</sup> and clinical nutrition products, e.g. Ensure<sup>®</sup> HN <sup>[24]</sup> have been suggested as media suitable for simulating fed state in the stomach.

For simulation of small intestine contents in both fasted and fed states, Dressman et al. <sup>[23]</sup> recently proposed the fasted state simulating intestinal fluid (FaSSIF) and the fed state simulating intestinal fluid (FeSSIF). In contrast to gastric media, these media contain bile salts and have pH values which mimic the small intestine environment. Also, their buffering capacity and osmolarity have been adjusted to simulate the average physiological values.

In this investigation SGF was used without Pepsin or any surfactant due to perturbation in size measurement as Pepsin is insoluble in this media (see table 2.4 in chapter 2). The effect of SGF in relation with water on the emulsification profile of Miglyol 812/Imwitor 988-Tagat TO system is presented in figure 3.1.17. The emulsification in both systems (water, SGF) has shown equivalent MEDD values for Miglyol 812-Tagat TO with no Imwitor 988 or with Imwitor 988 / Miglyol 812 ratio of 10/90.

Wakerly <sup>[6]</sup> in his equilibrium phase behaviour evaluation of the Tagat TO-Miglyol 812 system with 0.1M HCl found that the extent and quality of the LC<sub>a</sub> region was reduced comparable to the equivalent phase diagram with water. Yet, the level of effect on the LC<sub>a</sub> had not been significant at 37°C thus equivalence of MEDD data would be anticipated. However, this reduction in the quality of the LC<sub>a</sub> phase was attributed to the slightly reduced self-emulsifying performance in acid at a higher temperature, for example 45°C, or using high Tagat TO concentration (between 40 and 55%).



**Figure 3.1.17**

The Effect of self-emulsifying media on the self-emulsifying performance of different blends of Miglyol 812/Imwitor 988 using 30% (w/w) Tagat TO as measured by emulsion droplet diameter using QELS. Bars represent standard errors ( $n = 3$ ).

Nonetheless, as depicted in figure 3.1.17, the emulsification of oil systems containing Imwitor 988/Miglyol 812 at ratios of at least 20/80 and 30% surfactant in acid results in significant increase in the MEDD values in relation with water. Oils with Imwitor 988/Miglyol 812 ratios of 30/70 and 40/60 exhibited approximately 3-fold increase in the MEDD on emulsification in SGF.

Shinoda and Takeda <sup>[25]</sup> investigated thoroughly the effect of salts on the phase behaviour of nonionic surfactant system whereby a linear depression of phase boundary temperatures occurs across the whole phase diagram. The increase in the MEDD of oils with Imwitor 988/Miglyol 812 ratios of 30/70 and 40/60 can therefore be explained by a depression in the PIT of the system. Also a reduction in the water solubilization region ( $L_2$ ) would be anticipated in relation to the equivalent phase diagram with water. This suggests a phase inversion from o/w to w/o at temperatures lower than the preferable optimum temperature for emulsification (37°C). For use *in vivo* it would obviously be preferable to have optimum emulsification at 37°C yet this might be achieved by using slightly more hydrophilic surfactants such as, Cremophor RH 40 and Tween 80. Nonetheless, this does not preclude the use of this system *in vivo* since particles around 150nm are still achievable.

It is also worth noting that *in vitro* emulsification using SGF does not bring about a full picture of what is going on in the stomach. Hence the media excludes the human gastric lipase which initiates gastric lipolysis; a process that aids the emulsification as it facilitates the hydrolysis of triglycerides to diglycerides and fatty acids. It has recently been suggested <sup>[26]</sup> that a suitable surfactant be added to this media better simulate the surface tensions typical of the fasted stomach. However, *in vivo* studies are worth carried out to verify the performance of this genuine system in a suitable animal model.

### 3.1.2. Concluding Remarks

This present study was designed to develop stable o/w microemulsions comprising Miglyol 812 (medium chain,  $C_6$ - $C_{12}$ , triglycerides),  $C_8$ / $C_{10}$  mono/diglycerides (Imwitor 988) and non-ionic surfactant polyoxyethylene-25- glyceryl trioleate (Tagat

TO). This type of formulation is referred to as type II and is likely to retain its solvent capacity for the drug after dispersion as it is comprised of water in-soluble components. The resulting emulsions as assessed by particle size analysis showed to be dependent on the glycerides blend {Miglyol 812/Imwitor 988}, surfactant concentration, emulsification temperature and media. Manipulation of these parameters can result in emulsion formulations of controlled droplet size and hence surface area. Such considerations are important when the partition of lipophilic drugs into the aqueous phases and drug release rates are considered.

Miglyol 812-Tagat TO system exhibits optimum self emulsifying behaviour (MEDD of  $215.30 \pm 11.30$  nm) at 30°C. Nonetheless, Self-emulsifying oil in water (o/w) microemulsions has been obtained by incorporating medium-chain glycerides, particularly C<sub>8</sub>/C<sub>10</sub> mono/diglycerides (Imwitor 988). An optimized formulations consisting of Miglyol 812/Imwitor 988 (7:3) or (6:4) using 30% (w/w) Tagat TO has spontaneously emulsified in water at 37°C with gentle agitation, producing dispersions with mean droplet diameters of ~50 and 60 nm, respectively. These systems appeared to be affected by emulsification in the presence of electrolytes yet this has to be further investigated in vivo to establish whether there is a significant difference in bioavailability between systems which produce sizes of 250 versus 50nm.

Equilibrium phase behaviour studies have shown that good self-emulsification observed in Miglyol 812-Tagat TO system is related to the formation of lamellar liquid crystalline dispersion phase. For Miglyol 812-Tagat TO systems containing at least 3 parts of Imwitor 988, no liquid crystalline phase is involved in the mechanistic processes. However, the amount of water solubilized as w/o (L<sub>2</sub> phase) is of paramount importance for the observed good emulsification in these systems.

## **3.2. The Choice of Hydrophilic Surfactants for Formulation of Type III Self-micro-emulsifying Drug Delivery Systems (SMEDDS)**

### **3.2.1. Results and Discussion**

Microemulsions as drug delivery systems are designed to improve the drug solubilization and protection against enzymatic hydrolysis, as well as the potential for enhanced absorption. The exact mechanism(s) by which lipid enhances absorption of hydrophobic drug molecules is not fully understood, but is believed to involve transfer into the bile-salt mixed-micellar phase, from which absorption across the intestinal epithelium readily occurs.

The choice of a suitable microemulsion system for drug delivery requires substantial knowledge about the physicochemical properties of the microemulsion system such as miscibility of the oil mixture, drug solubility in the pre-concentrate, phase equilibrium behaviour on dilution with water and particle size of the resulting dispersions.

Cyclosporin A which is an immunosuppressant for organ transplant patients has been reformulated as a microemulsion system. The advent of Neoral<sup>®</sup> (Novartis) has given new impetus to this evolving technology as an important approach to improve the oral absorption and thus enhance the bioavailability of hydrophobic active molecules. A full literature search reveals well over a hundred recent publications on the formulation, biopharmaceutics and pharmacokinetics of cyclosporin A [27]. The previous formulation of Cyclosporin was an oil-based solution called Sandimmun<sup>®</sup> which, after oral administration, formed crude o/w dispersion which had to be exposed to local secretions of lipase and bile salts from the pancreas and gall-bladder respectively, in order to facilitate the digestion of the oil droplets and therefore the release of Cyclosporin. Neoral<sup>®</sup>, on the other hand, readily forms a microemulsion on contact with the gastrointestinal fluids without the involvement of lipolysis, and as a consequence the bioavailability and reproducibility of delivery of cyclosporin is substantially enhanced.

The optimization of cyclosporin as Neoral<sup>®</sup> is the archetypal example of Type IIIB according to the classification system proposed by Pouton [5]. The Neoral<sup>®</sup> formulation uses approximately 20% co-solvent, 30% oil and 40 % hydrophilic

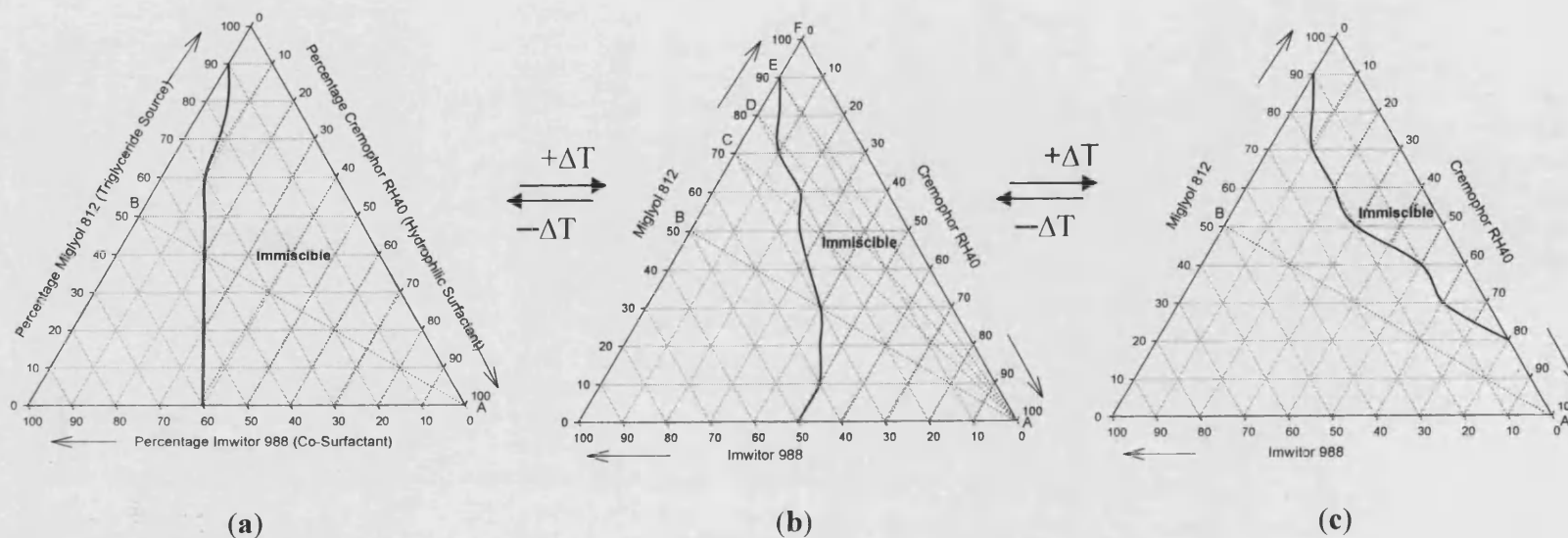
nonionic surfactant (Cremophor RH40) and 10% drug. Apparently, the use of Cremophor RH40 (polyoxyethylene-(40)-hydrogenated castor oil) has significantly contributed to the success of this formulation as a microemulsion delivery system. Yet the role of this surfactant in the emulsification process from mechanistic and phase behaviour perspective has not been reported.

In this study blends of Miglyol 812, Imwitor 988 and Cremophor RH40 were optimized to produce microemulsions. The effect of varying the weight ratio of each component on the emulsification performance for various microemulsion systems as determined by size measurement was investigated. Equilibrium phase behaviour study is also conducted to establish the mechanism by which Cremophor RH40 influences the emulsification process.

Hence Cremophor RH40 is a waxy material at room temperature as it has a high melting point of  $\approx 40^{\circ}\text{C}$ ; it restricts the range of oil mixtures which can be used in the system. It also implicates the scale-up production of lipid formulations as special considerations are needed to handle and process a molten formulation on automated equipment. Solidification of the lipid system and thus drug precipitation can also occur during processing and storage due to variations in the environmental temperature. Therefore, in order to circumvent the crystallization of monoglycerides and/or highly ethoxylated surfactant molecules, alternative hydrophilic surfactants were investigated here for microemulsion systems.

#### 3.2.1.1. Miscibility of Ternary Oil Systems

Figure 3.2.1(a, b, and c) depicts ternary phase diagrams displaying the miscibility of Miglyol 812/Imwitor 988-Cremophor RH40 system at various storage temperatures  $15^{\circ}\text{C}$ ,  $25^{\circ}\text{C}$  and  $45^{\circ}\text{C}$ , respectively. This system contains two pairs of partially miscible liquids at temperatures  $15^{\circ}\text{C}$  and  $25^{\circ}\text{C}$ , viz Cremophor RH40 and Imwitor 988 also Cremophor RH40 and Miglyol 812. Yet, it is important to note that blends of Cremophor RH40 and Imwitor 988 might be completely miscible and what we see from the area of immiscibility close to the Imwitor 988 line is due to the solidification of Cremophor RH40 at temperatures below the melting point ( $40^{\circ}\text{C}$ ). This is substantiated, however, as the area of immiscibility between Cremophor RH40 and Imwitor 988 has disappeared when the waxy Cremophor RH40 is replaced with its



**Figure 3.2.1:**

The phase equilibrium in a three-component system for Miglyol 812/Imwitor 988-Cremophor RH40 formulation under various isothermal conditions: (a) 15°C (b) 25°C (c) 45°C.



liquid unsaturated form Cremophor EL, see figure 3.2.2. At 45°C, however, only Cremophor RH40 and Miglyol 812 show partial miscibility as they form one bimodal curve. On the other hand, Miglyol 812 and Imwitor 988 are completely miscible at all temperatures used. Generally, increasing the temperature leads to a reduction in the areas of immiscibility and thus miscibility is promoted. The line A-B depicted in figure 3.2.1(a, b and c) represents the dilution of Miglyol 812/Imwitor 988 ratio of 5:5 with increasing concentration of Cremophor RH40. It shows that the maximum amount of Cremophor RH40 that can be used to formulate isotropic blends is 20%, 40% or up to 90% at temperatures 15°C, 25°C and 45°C, respectively. This suggests that reduction of the temperature expands the area of immiscibility and prompts crystallization of Cremophor RH40 which could result in drug precipitation. This nullifies the purpose of lipid formulations which are designed to improve the dissolution rate of poorly water-soluble drugs and thus enhance absorption. Therefore, as it has been shown, the choice of Cremophor RH40 limits the range of acceptable oil formulations that can be used to produce microemulsion systems. The use of co-solvent in these systems might improve the range of reliable oil systems. Yet this, however, raises the issue of losing the solvent capacity of these formulations on dilution of the co-solvent in the lumen of the gut which might ensue in crystallization of the drug. Alternatively, to screen other surfactants which could replace Cremophor RH40; in particular the liquid surfactants Cremophor EL and polysorbate 80.

Figure 3.2.2 shows the phase equilibrium in a three component system of Miglyol 812/Imwitor 988 and Cremophor EL (polyoxyethylene-(35)-caster oil). In contrast to the oil systems containing Cremophor RH 40, this ternary phase diagram displays a huge area of miscibility, in which Cremophor EL and Miglyol 812 show partial miscibility as there is only one bimodal curve. The phase diagram depicted in figure 3.2.2 reveals similar areas of phase equilibrium as the ternary oil mixtures which contain Cremophor RH 40 at 45°C (Figure 3.2.1c). This suggests that what causes the crystallization of these systems containing Cremophor RH40 and thus restricting the range of oil systems which can be used is the waxy nature of the surfactant. Especially, if we know that Cremophor EL which is obtained by reacting ethylene oxide with caster oil in a molar ratio of 35 moles to 1 mole, is the liquid form of Cremophor RH40 (polyoxyethylene-(40)-hydrogenated caster oil) with slightly lower HLB values (HLB, between 12 and 14) as it contains less ethoxy residues per

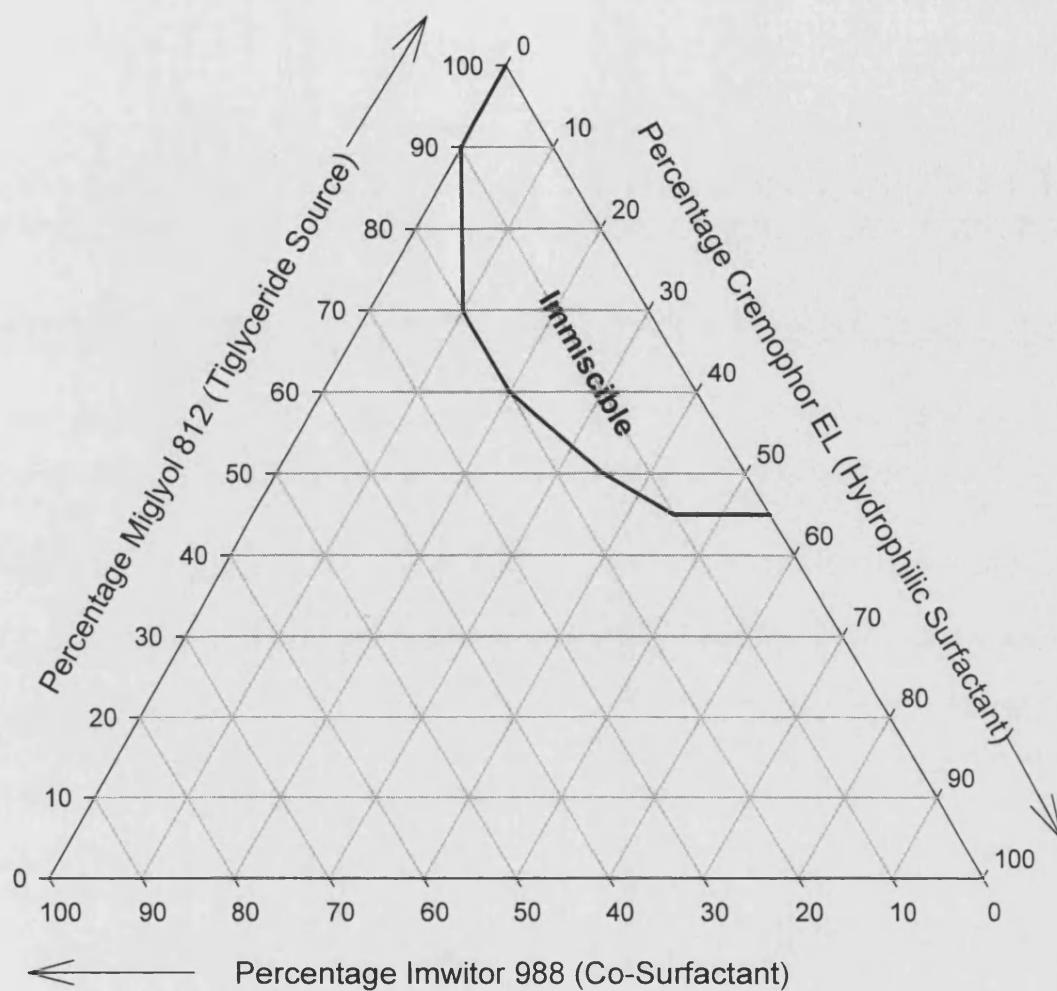


Figure 3.2.2

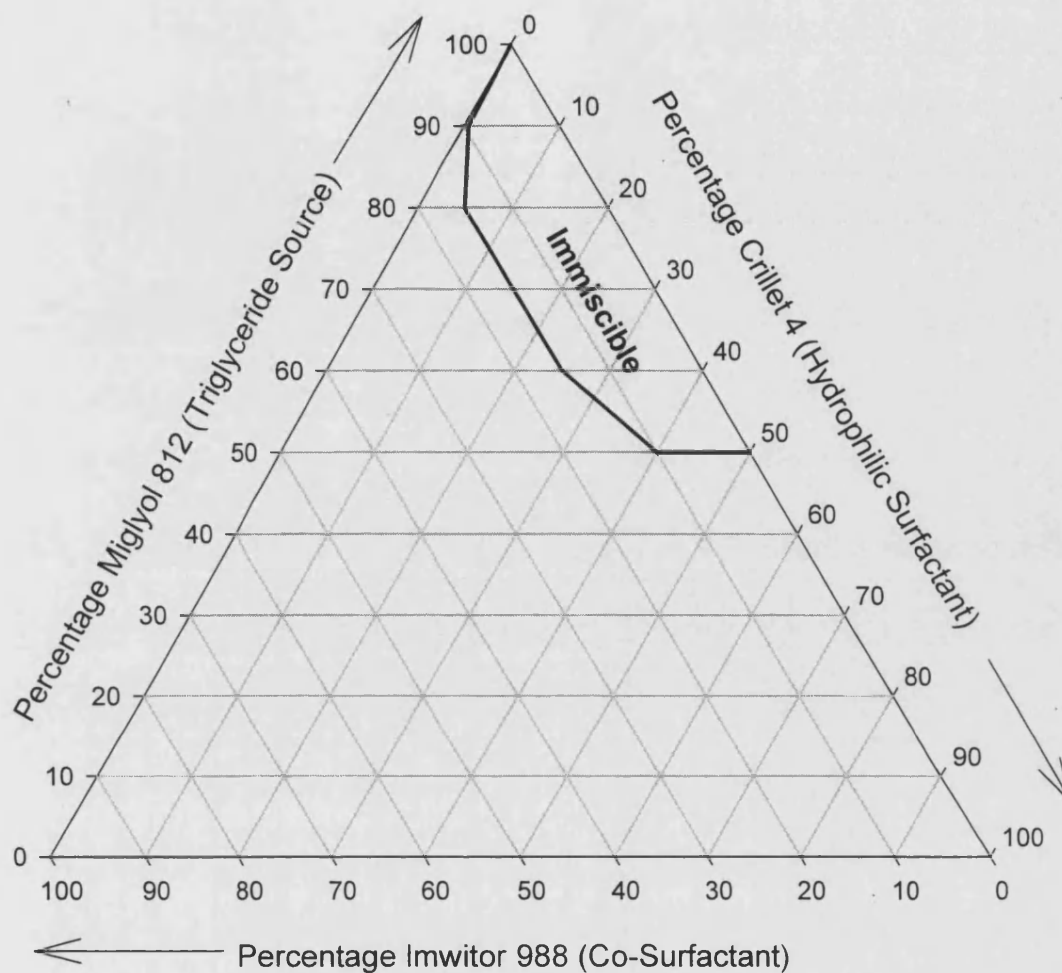
Ternary phase diagram for Miglyol 812/Imwitor 988-Cremophor EL system displaying miscibility for formulations of various compositions.

molecule. Therefore, comparable MEDD profiles would be anticipated from emulsification of oil systems containing either Cremophor RH 40 or EL.

The ternary phase equilibrium diagram for Miglyol 812, Imwitor 988 and Crillet 4 (Polyoxyethylene-(20)-sorbitan monooleate) displayed in figure 3.2.3 has produced similar phase miscibility as oil systems containing Cremophor EL depicted in figure 3.2.2. Crillet 4 and Miglyol 812 show partial miscibility as there is only one bimodal curve, Crillet 4 and Imwitor 988, on the other hand, are completely miscible. This could be due to the fact that both surfactants have approximately equivalent HLB values. Nonetheless, it is surmised that oil systems containing either Crillet 4 or Cremophor EL will produce different emulsification profiles on dilution with water as both surfactants have different chemical entities which will be elaborated further in next sections.

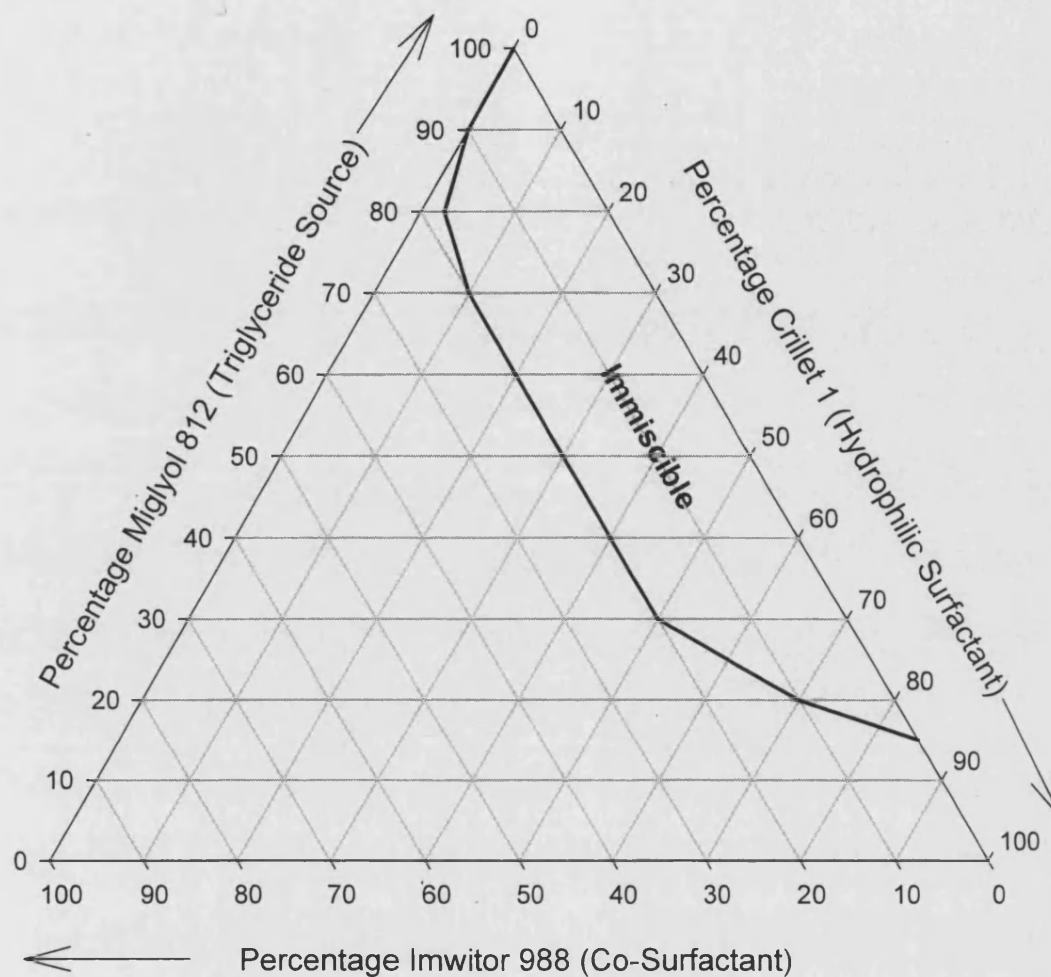
Replacing Crillet 4 (HLB =15) with a surfactant of higher HLB value (Crillet 1, HLB =16.7) has expanded the bimodal curve, see figure 3.2.4. Crillet 1 (Polyoxyethylene-(20)-sorbitan monolaurate) is considered to be more hydrophilic than Crillet 4 as the former contains lower chain fatty acid derivative (Lauric acid  $C_{12:0}$ ) while the latter has an oleic acid ( $C_{17:2}$ ) residue. The extension of the area of immiscibility observed in figure 3.2.4 vis-à-vis the ternary phase equilibrium for oil systems containing Crillet 4 (figure 3.2.3) is attributed to the relatively high HLB value of Crillet 1 due to the decrease of the molecular weight of the fatty acid residue which reduces the solubility in oil and increases aqueous solubility.

This is more evident in the case of using Tagat O2 (polyoxyethylene-(20)-glyceryl monooleate, HLB =15) in the three-component system depicted in figure 3.2.5. From one hand, it has produced similar bimodal curve as oil systems containing surfactants with comparable HLB values; Cremophor EL or Crillet 4 (Figures 3.2.2 and 3.2.3, respectively). On the other hand, the ternary oil system containing Miglyol 812, Imwitor 988 and Tagat TO (HLB =11) has shown area of total miscibility (figure 3.1.1 in the previous section) while using a more hydrophilic surfactant (Tagat O2) a bimodal curve has appeared. This reflects the fact that Miglyol 812 and Tagat TO are completely miscible whereas Miglyol 812 and Tagat O2 show partial miscibility. This is attributed to the differences in the HLB values for both surfactants, in which Tagat O2 is more hydrophilic as it is based on monooleate fatty acid ester while Tagat TO is a trioleate ester.



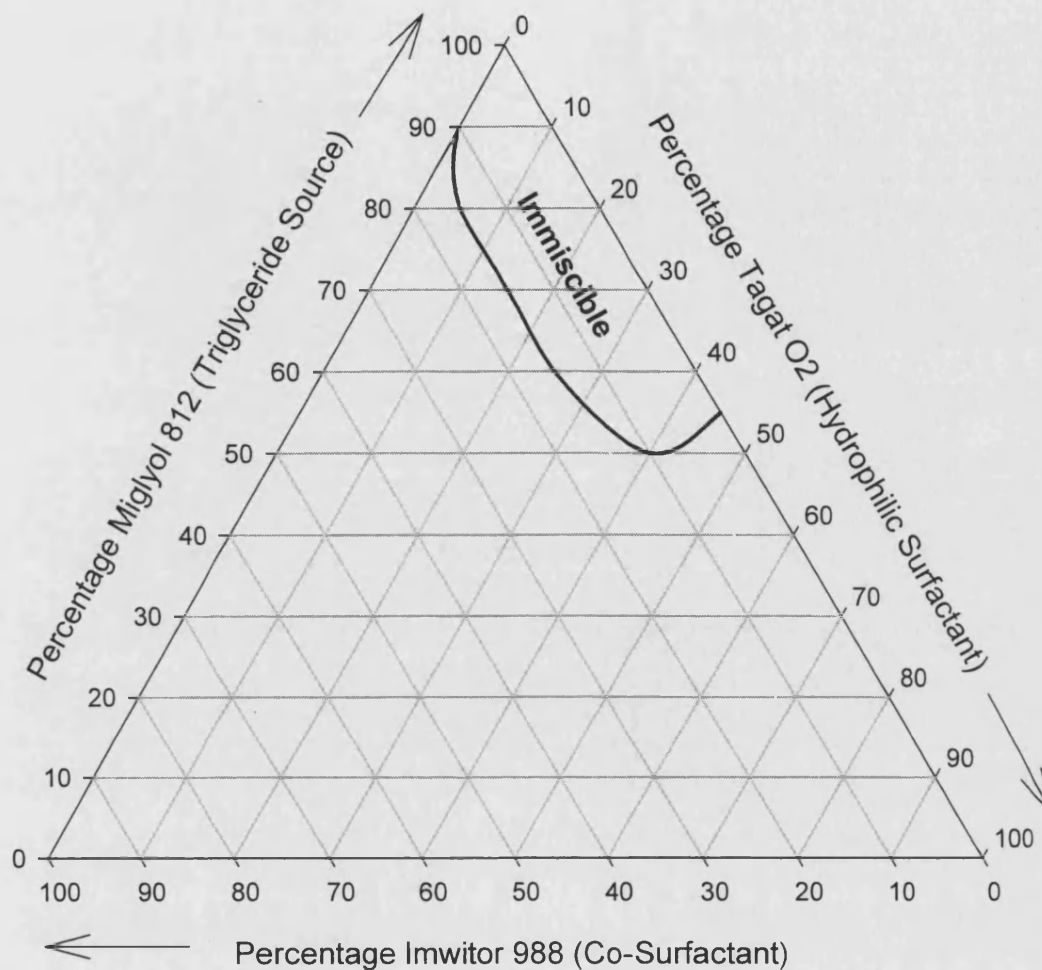
**Figure 3.2.3**

Ternary phase diagram for Miglyol 812/Imwitor 988-Crillet 4 system displaying miscibility for formulations of various compositions.



**Figure 3.2.4**

Ternary phase diagram for Miglyol 812/Imwitor 988-Crillet 1 system displaying miscibility for formulations of various compositions.



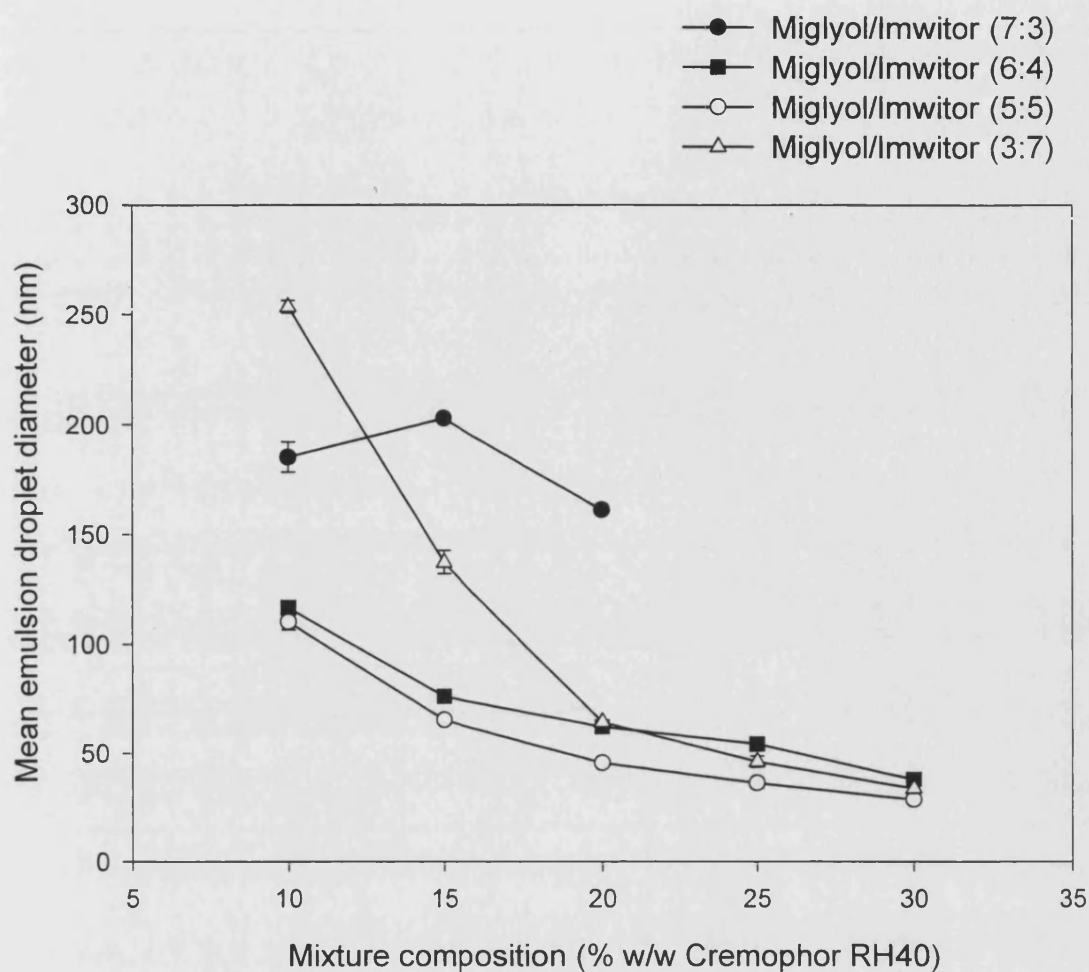
**Figure 3.2.5**

Ternary phase diagram for Miglyol 812/Imwitor 988-Tagat O2 system displaying miscibility for formulations of various compositions.

### 3.2.1.2. Emulsification Performance of Oil Systems

*In vitro* particle size distribution analysis is one of the most important means to evaluate the stability of emulsions and also its *in vivo* fate after oral administration of the pre-concentrate, that is, a drug-surfactant-oil mix. Figures 3.2.6, and 7 display the effect of surfactant concentration and oil blends of Miglyol 812/Imwitor 988 on the emulsification performance of Miglyol 812/Imwitor 988-Cremophor RH40 system as determined by the mean emulsion droplet diameter (MEDD). At first, oil systems containing Miglyol 812, Miglyol 812/Imwitor (9:1) or (8:2) blended with Cremophor RH40 show either complete immiscibility or isotropic single phase oil mixtures with very limited concentration of Cremophor RH40 <10% w/w (figure 3.2.1b, lines A-F, A-E and A-D). Therefore, these oil mixtures were excluded from the emulsification study. On the other hand, for oil blends of Miglyol 812/Imwitor (7:3), Cremophor RH40 was included at concentration below 25% w/w lest using more surfactant in the system will ensue in phase separation due to crystallization, see figure 3.2.1b (line A-C).

Generally, as depicted in figure 3.2.6, the mean emulsion droplet diameter for Miglyol 812/Imwitor 988-Cremophor RH40 system decreased with increasing surfactant concentration at all Miglyol 812/Imwitor 988 ratios. Such a decrease in droplet size may be due to the availability of more surfactant to stabilize the oil-water interface. Furthermore, the decrease in the droplet size behaviour reflects the formation of a better close packed film of the surfactant at oil-water interface which stabilizes the oil droplets<sup>[28]</sup>. Yet, the emulsification of Miglyol 812/Imwitor 988 blends of 7:3 ratio at surfactant content up to 20% w/w, which is the maximum concentration to avoid crystallization, did not affect the droplet size significantly and thus produced dispersions with high MEDD values. However, with increasing the cosurfactant content (Imwitor 988) in the Miglyol 812/Imwitor 988-Cremophor RH40 system, the droplet size decreased substantially. This suggests that increasing the Imwitor 988 content in the system reduces the surfactant concentration which produces comparable MEDD values. For example, Miglyol 812/Imwitor 988 (6:4) at 20% w/w Cremophor RH40 has produced a dispersion with MEDD of  $\approx 62\text{nm}$  yet, only 15% w/w of Cremophor RH40 is needed to obtain equivalent droplet size ( $\approx 65\text{nm}$ ) when Miglyol 812/Imwitor 988 is blended at 5:5 ratio. This effect is up to a point beyond which further increment of the co-surfactant would increase the MEDD values and thus



**Figure 3.2.6**

Mean emulsion droplet diameter (MEDD) profiles for self-emulsified systems containing different ratios of Miglyol 812/Imwitor 988 and increasing concentration of Cremophor RH40. Lipid formulations were emulsified in water at 37°C for 15 minutes. MEDD values were measured by photon correlation spectrometer (PCS). Bars Represent Standard Errors (n = 3).

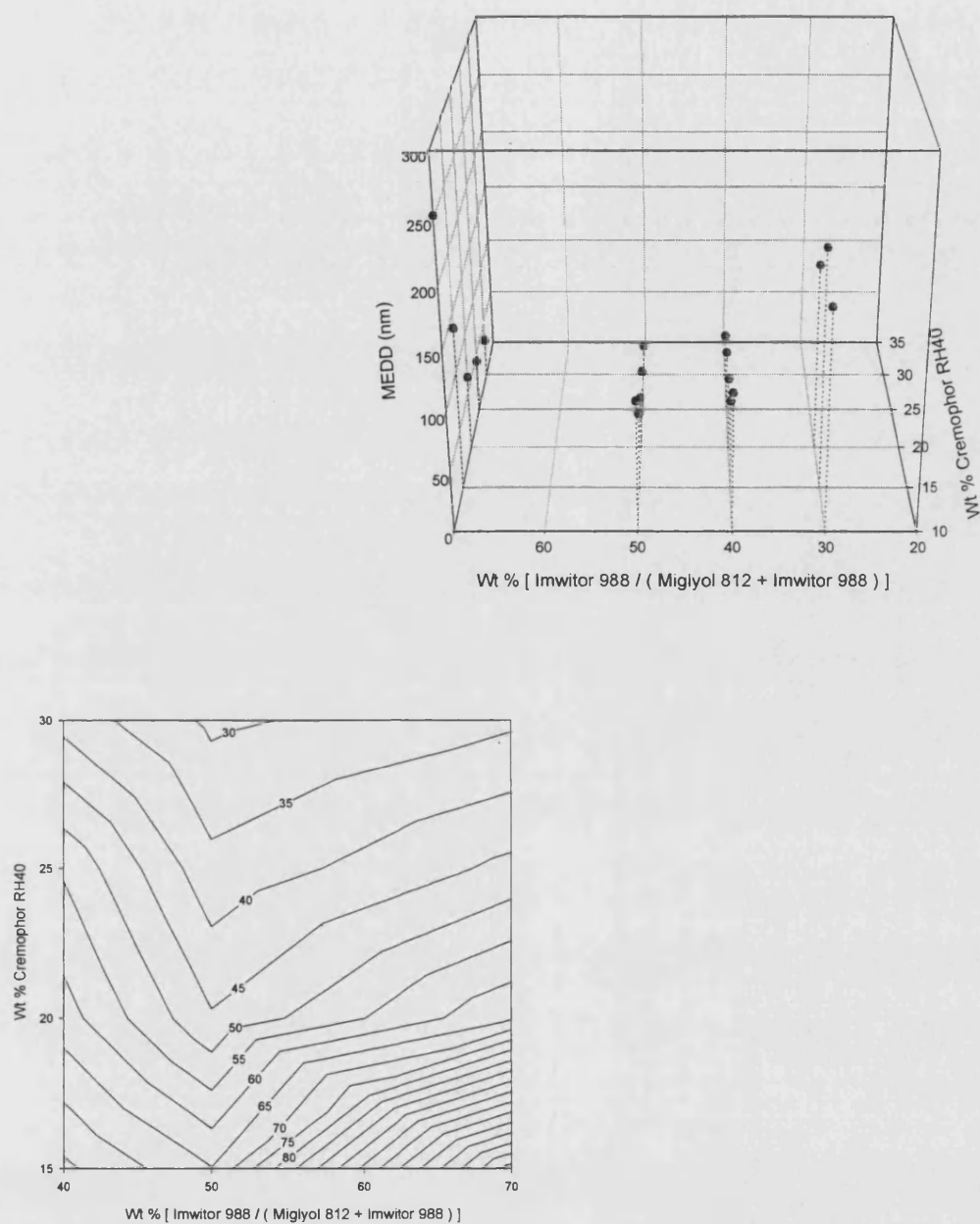


more surfactant is needed to obtain equivalent MEDD values. In order to illustrate that, the emulsification of Miglyol 812/Imwitor 988 (3:7) at 15% w/w Cremophor RH40 produced a dispersion with MEDD of  $\approx 137\text{nm}$  however, when the system had only 5 parts of Imwitor 988 at same surfactant concentration, MEDD of 65nm was achieved. Furthermore, for the Miglyol 812/Imwitor 988 (3:7) system to obtain approximately similar MEDD as 5:5 formulation (65nm), 20% w/w of Cremophor RH40 is needed.

The emulsification profile of Miglyol 812/Imwitor 988 (6:4)-Cremophor RH40 system is comparable to 5:5 system yet, relatively lower MEDD values were obtained in the later. The emulsification of Miglyol 812/Imwitor 988 (5:5)-Cremophor RH40 system produced optically clear dispersions at surfactant concentration  $>20\%$  w/w, minimum MEDD value around 30 nm was achieved at 30% w/w Cremophor RH40. On the other hand, Miglyol 812/Imwitor 988 ratios of 6:4 and 3:7 produced microemulsions at higher surfactant concentration  $\geq 30\%$ . This indicates that regardless Miglyol/Imwitor 988 ratio in the oil blends, microemulsions can still be obtained provided higher surfactant concentration is used. Yet, this raises a serious problem of damage to the gastrointestinal mucosa, as these systems contain a relatively large amount of surfactants.

The three-dimensional graph with contour plot for the emulsification performance of Miglyol 812/Imwitor 988-Cremophor RH40 system (Figure 3.2.7) shows an optimum blend of Miglyol 812/Imwitor 988 at 1:1 ratio which has produced the finest dispersions. A decrease in MEDD was observed with an increase in cosurfactant concentration from 30% to 50%, after which the MEDD was slightly increased. Similar observations have been reported <sup>[29, 30]</sup> where further increase in cosurfactant concentration increased the droplet size. This was demonstrated by Gao et al. <sup>[30]</sup> using microemulsion systems containing Captex-355 as an oil, Cremophor EL as a surfactant, Transcutol as a cosurfactant and saline.

It is important to note here that the need for a cosurfactant is related to the fact that each head group unit is hydrophilic. Therefore, the addition of an extra unit considerably increases the hydrophilicity of the surfactant and hence, it is made harder to achieve the correct balance of the hydrophobic and hydrophilic portions of the surfactant required for production of a microemulsion; instead this balance has to



**Figure 3.2.7**

Three-dimensional graph and contour plot representations of MEDD data for the emulsification of Miglyol 812/Imwitor 988-Cremophor RH40 system as a function of surfactant concentration and ratio of Imwitor 988/Miglyol 812 in the oil blend.

be achieved by the use of a cosurfactant <sup>[35]</sup>. The addition of surfactant to the microemulsion systems causes the interfacial film to condense and to be stable, while the addition of cosurfactant causes the film to expand <sup>[36]</sup>. When a cosurfactant is added to the system with the surfactant, it lowers the interfacial tension, fluidizes the hydrocarbon region of the interfacial film and decreases the bending stress of the interface <sup>[37]</sup>.

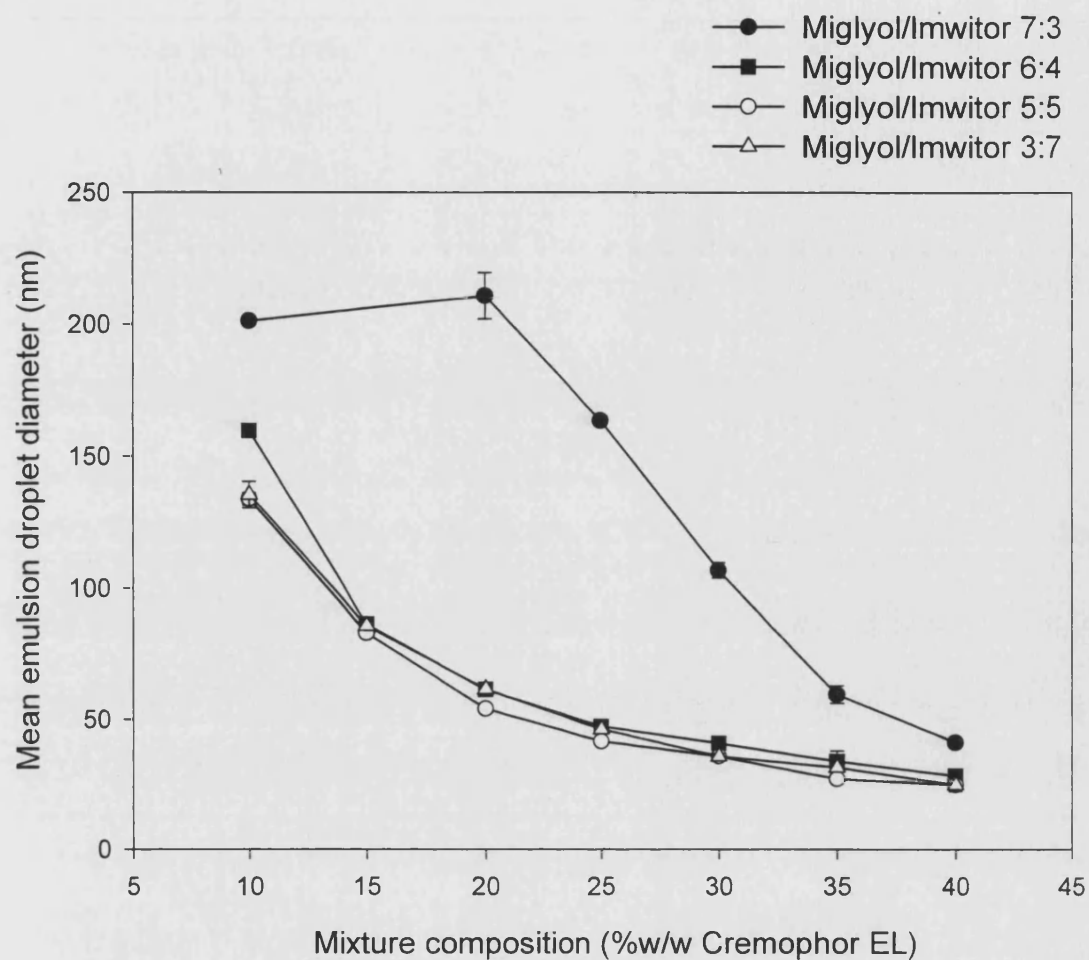
As Imwitor 988 is polar oil due to the glycerol mono- caprylate content ( $\approx 50\%$ ), the polarity of oil droplets is optimized at certain ratio of Miglyol 812/Imwitor 988 after which, any further increase in the Imwitor 988 content would affect the hydrophilic-lipophilic balance (HLB) of the system and consequently the emulsification is affected. However, in a study by Kawakami *et al* <sup>[31, 32]</sup> they concluded that surface tension and polarity measurements of oil blends did not correlate with the system ability to form microemulsions as determined by the amount of oil solubilized in the surfactant-water mixtures. They demonstrated that the most effective mixing ratio to mimic the solubilization behaviour was 1:1 of glycerol monocaprylic ester (MCG) and propyleneglycol dicaprylic ester (DCPG), although the later was hardly solubilized in any surfactant solutions. Yet, they offered one possible assumption whereby the DCPG phase may be enclosed in a shell of the MCG rich phase hence MCG molecules may penetrate into the surfactant layer as the penetration of polar oils into surfactant layer has been observed <sup>[33, 34]</sup>. This will be reflected upon when we study the mechanistic processes involved in the emulsification of Miglyol 812/Imwitor 988-Cremophor RH40 system. On the other hand, in this investigation the emulsification of Imwitor 988 (Glycerol mono-/di-caprylate {1:1})-Cremophor RH40 system (i.e. without any Miglyol 812) produced dispersions with inconsistent and high MEDD values which suggest the need to include medium chain triglyceride (MCT) in the formulation. Apparently, this contrasts results from Kawakami *et al* <sup>[31]</sup> in the case of Cremophor RH40 whereby maximum solubilization occurred at MCG/DCPG ratio of 1:1. This could be attributed to the fact that in their study the solubilization behaviour of ternary oil mix MCG, DCPG and MCT was not optimized as they investigated these ester oils each one alone or as a binary mix. Also, they have used one surfactant concentration that is, 10% w/w which appears to be very small when considering forming o/w microemulsions.

In an attempt to prevaricate the crystallization tendency of Cremophor RH40 in the pre-microemulsion concentrate and thus expands the range of mixtures which can be used, alternative surfactants were investigated such as, Cremophor EL, Crillet 4, Crillet 1 and Tagat O2.

The emulsification profiles with contour plot for Miglyol 812/Imwitor 988-Cremophor EL is depicted in figures 3.2.8 and 9. Cremophor EL (polyoxyethylene-(35)-caster oil) is considered to be the liquid form of Cremophor RH40 (HLB, 14-16) yet with lower HLB value (12-14) due to less ethoxylation number. This has reflected on the area of miscibility as depicted by the ternary phase diagram (Figure 3.2.2) which offers wide range of potential pre-microemulsion mixtures without restriction on the amount of surfactant to be included. As illustrated in figure 3.2.8, the emulsification profiles of Miglyol 812/Imwitor 988-Cremophor EL have shown trends similar to formulations containing Cremophor RH40 whereby droplet size is controlled by the surfactant concentration and the oil-cosurfactant ratio. With increasing the amount of surfactant or cosurfactant in the system, smaller MEDD could be obtained. Yet, the increase in the cosurfactant-oil ratio is up to a point beyond which the polarity of oil droplets is enhanced and thus the HLB of the oil system becomes out of the range to produce microemulsions and consequently the MEDD is increased.

As figure 3.2.8 depicts, the emulsification performance of Miglyol 812/Imwitor 988 (7:3) using Cremophor EL has produced dispersions with high MEDD values. This system at surfactant concentration between 10 and 20%w/w, has produced comparable MEDD values to formulations containing same oil blend (7:3) but using Cremophor RH40 (Figure 3.2.6). It is worth noting here that, the 7:3 oil blends at Cremophor RH40 concentration of > 20% w/w produced unstable pre-concentrate formulations.

Moreover, the emulsification profiles of Miglyol/Imwitor (6:4), and (5:5) at Cremophor EL concentration of  $\geq 15\%$  w/w (Figure 3.2.8) produced comparable MEDD profiles to same oil blends but containing Cremophor RH 40 (Figure 3.2.6). Interestingly, in contrast to the variations in the emulsification profiles of Miglyol 812/Imwitor 988 (6:4), (5:5) and (3:7) using Cremophor RH40 (Figure 3.2.6), these systems have shown equivalence of MEDD profiles in the case of Cremophor EL at concentration of  $\geq 15\%$  w/w (Figure 3.2.8). This is evident from the three dimensional



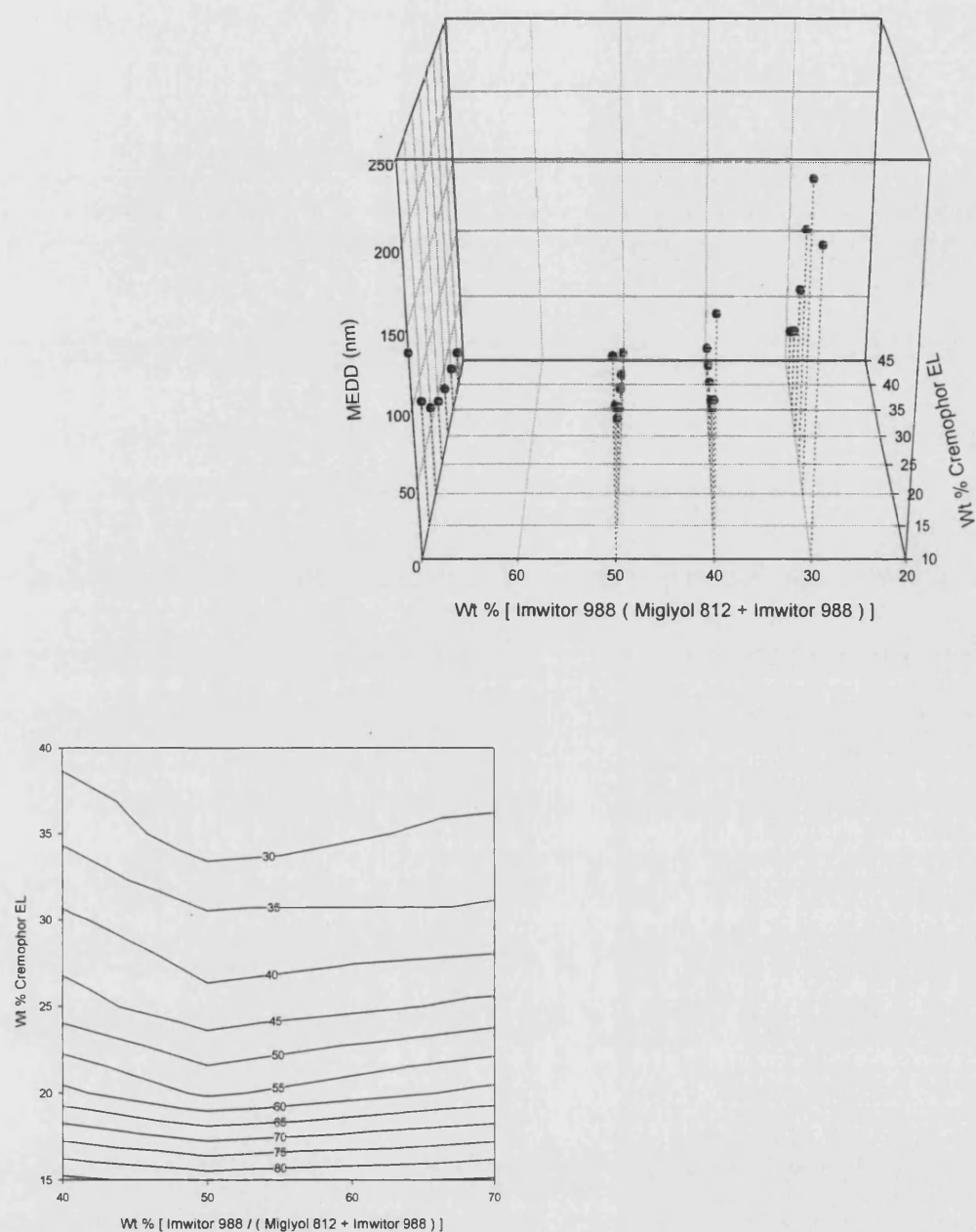
**Figure 3.2.8**

Emulsification profiles of self-emulsified systems containing different ratios of Miglyol 812/Imwitor 988 and increasing concentration of Cremophor EL. Lipid formulations were emulsified in water at 37°C for 15 minutes. MEDD values were measured by photon correlation spectrometer (PCS). Bars Represent Standard Errors (n = 3).

and contour representations of the MEDD data (Figure 3.2.9) viz increasing the amount of cosurfactant from 40-70 %w/w in the oil blend at Cremophor EL  $\geq 15\%$  w/w has produced dispersions with comparable MEDD values. This has reflected on the contour lines which have been almost flat with increasing the amount of cosurfactant specifically from 50-70% w/w. Unlike the minimum trough observed in the contour representation of the MEDD for Miglyol 812/Imwitor 988-Cremophor RH40 system (Figure 3.2.6), Minimum MEDD of 30nm at approximately 35% w/w Cremophor EL has extended over a cosurfactant weight percentage from 50-70%. This broad range of cosurfactant amount in the oil blend which has produced microemulsion systems with no variations in the emulsification profiles is attributable to the relatively low HLB value of Cremophor EL comparing to RH 40. As discussed earlier, the use of cosurfactant normally adds to the total polarity of oil droplets and hence increases the HLB of the system. If the surfactant has already high HLB value as in the case of Cremophor RH40 (HLB between 14 and 16), the amount of cosurfactant that is needed to obtain an HLB value of the oil mix within the range for producing microemulsion systems is limited. However, for Cremophor EL which has relatively lower HLB value (between 12 and 14), the range of cosurfactant that is required to produce an oil mix with an HLB value which is within the range to obtain fine dispersions is widened.

It is important to note here that the emulsification of pure Imwitor 988 and Cremophor EL has produced bad quality dispersions with high MEDD values. This suggests that the cosurfactant weight percentage of 70% in the oil blend might be the maximum amount for obtaining pre-microemulsion concentrate.

Last but not the least, provided optimum oil blends are used, Cremophor EL offers a good alternative to Cremophor RH40 for formulation of SMEDDS (see table 3.2.1). In a study by Gao et al. <sup>[30]</sup> using Captex 355<sup>®</sup> as an oil, Cremophor EL<sup>®</sup> as a surfactant, Transcutol<sup>®</sup> as a cosurfactant and saline, microemulsion systems were optimized at Cremophor EL<sup>®</sup>:Transcutol<sup>®</sup>:Captex 355<sup>®</sup>, 10:5:4 i.e. oil-cosurfactant ratio of approximately 1:1 with 50% surfactant. Though the oil-cosurfactant ratio of the oil blend agrees with our findings yet the use of high content of surfactant in his system raises serious toxicity issues.



**Figure 3.2.9**

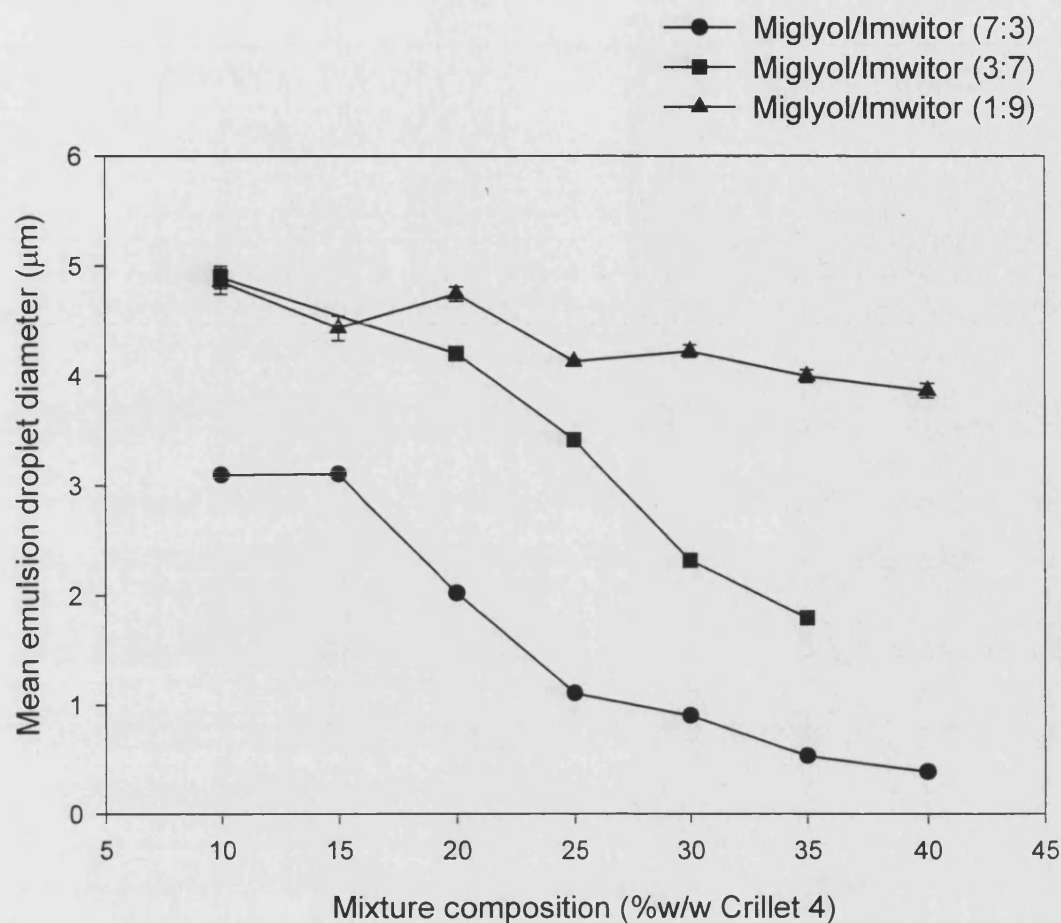
Three-dimensional graph and contour plot representations of MEDD data for the emulsification of Miglyol 812/Imwitor 988-Cremophor EL system as a function of surfactant concentration and ratio of Imwitor 988/Miglyol 812 in the oil blend.

**Table 3.2.1: Microemulsions prepared by various oil-cosurfactant-surfactant systems.**

Formula	Miglyol/Imwitor (5:5)- Cremophor RH40	Miglyol/Imwitor (5:5)- Cremophor EL	Miglyol/Imwitor (4:6)- Crillet 4
Surfactant Wt %	MEDD (nm)		
10	110.40±3.70	133.80±2.30	Off range
15	65.25±0.21	83.00±0.42	Off range
20	45.55±0.78	54.00±1.60	220.33±4.65
25	36.50±2.40	41.50±0.57	75.10±0.40
30	28.95±1.20	35.90±0.99	38.80±1.00
35	27.2±0.00	27.20±0.00	32.80±0.20
40	unstable	25.00±2.50	28.00±1.30

Figures 3.2.10, 11 and 12 display the emulsification performance of Miglyol 812/Imwitor-Crillet 4 (polyoxyethylene(20) sorbitan monooleate) system as analysed by the effect of surfactant concentration and oil-cosurfactant ratio on the droplet size. The emulsification of Miglyol 812/Imwitor 988 (7:3), (3:7) and (3:9)-Crillet 4 have produced dispersions with MEDD values between 1 and 5  $\mu\text{m}$  (Figure 3.2.10). These formulations according to their droplet size distribution are classified as self-emulsified drug delivery systems (SEDDS). Nonetheless, oil blends containing an amount of Imwitor 988 between 40% and 60% produced fine dispersions and therefore were further investigated using the PCS (Figure 3.2.11). Similar to oil systems containing either Cremophor RH40 or EL (Figures 3.2.6 and 8, respectively); increasing the surfactant concentration or the cosurfactant to oil ratio in the Miglyol 812/Imwitor 988-Crillet 4 system reduces the MEDD values. Yet, the increase of the cosurfactant in the oil blend is also to a point beyond which further increment would ensue in increase in the MEDD values. As the three dimensional graph with the contour plot for Miglyol 812/Imwitor 988-Crillet 4 system depict (Figure 3.2.12), microemulsion systems are optimised at Miglyol 812/Imwitor 988 ratio of 4:6. The contour plot representation of the MEDD data for this system shows minimum trough





**Figure 3.2.10**

Mean emulsion droplet diameter (MEDD) profiles for self-emulsified systems containing different ratios of Miglyol 812/Imwitor 988 and increasing concentration of Crillet 4. Lipid formulations were emulsified in water at 37°C for 15 minutes. MEDD values were determined by laser diffraction method using the Mastersizer. Bars Represent Standard Errors (n = 3).

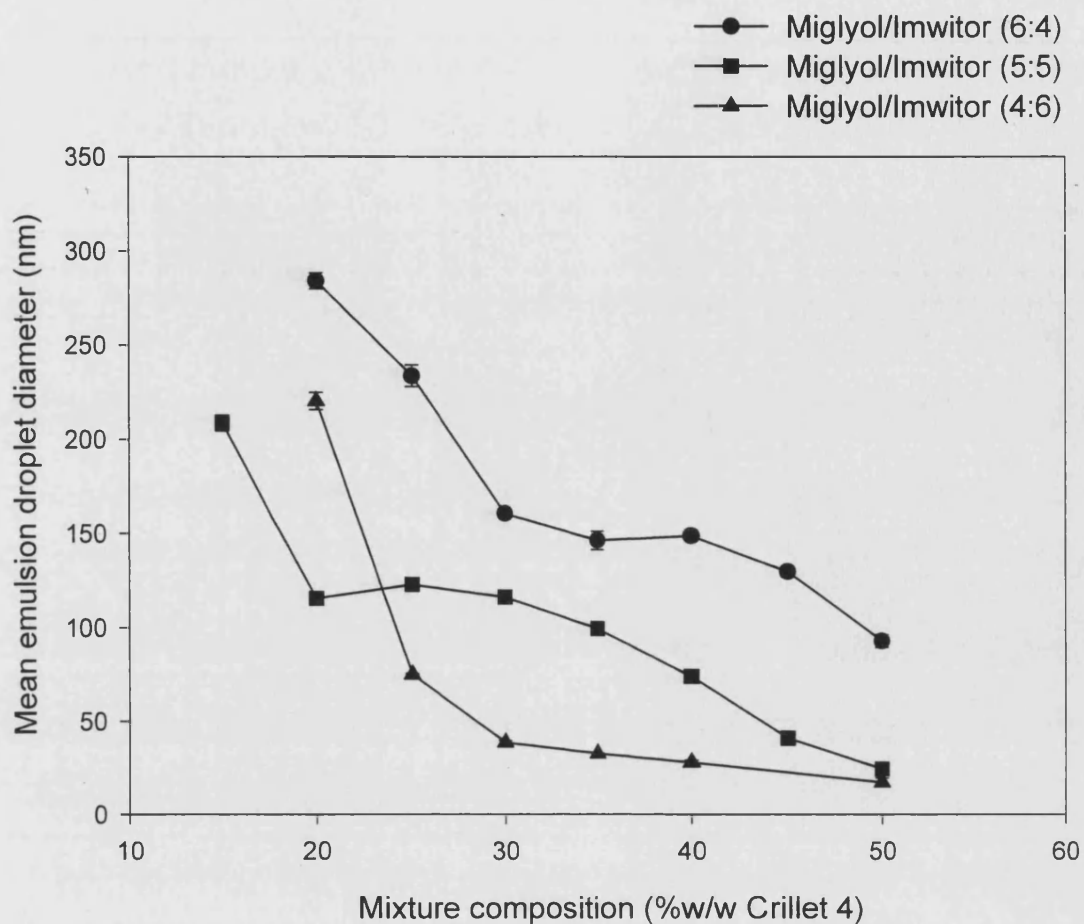
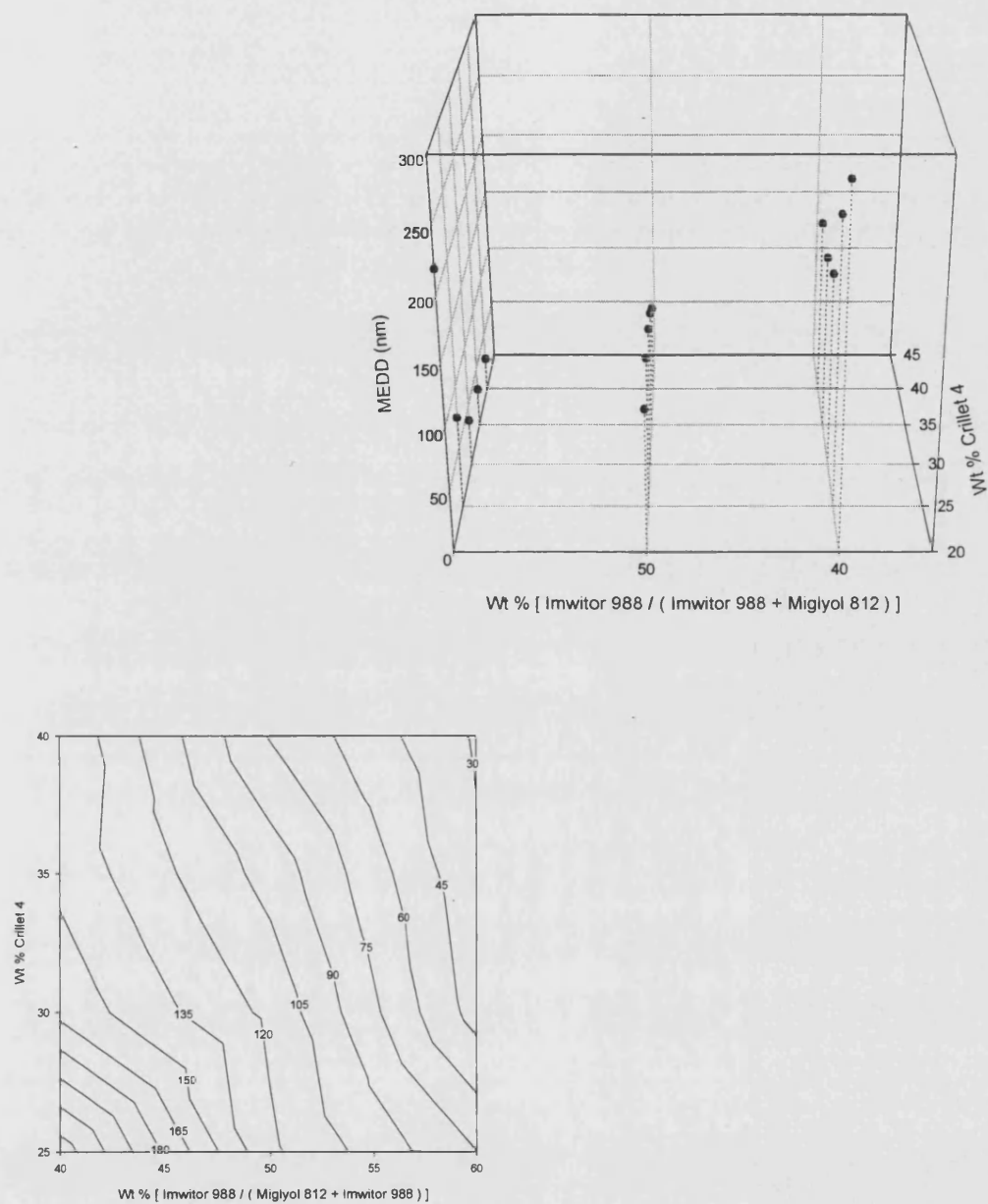


Figure 3.2.11

Emulsification profiles of self-emulsified systems containing different ratios of Miglyol 812/Imwitor 988 and increasing concentration of Crillet 4. Lipid formulations were emulsified in water at 37°C for 15 minutes. MEDD values were measured by photon correlation spectrometer (PCS). Bars Represent Standard Errors (n = 3).



**Figure 3.2.12**

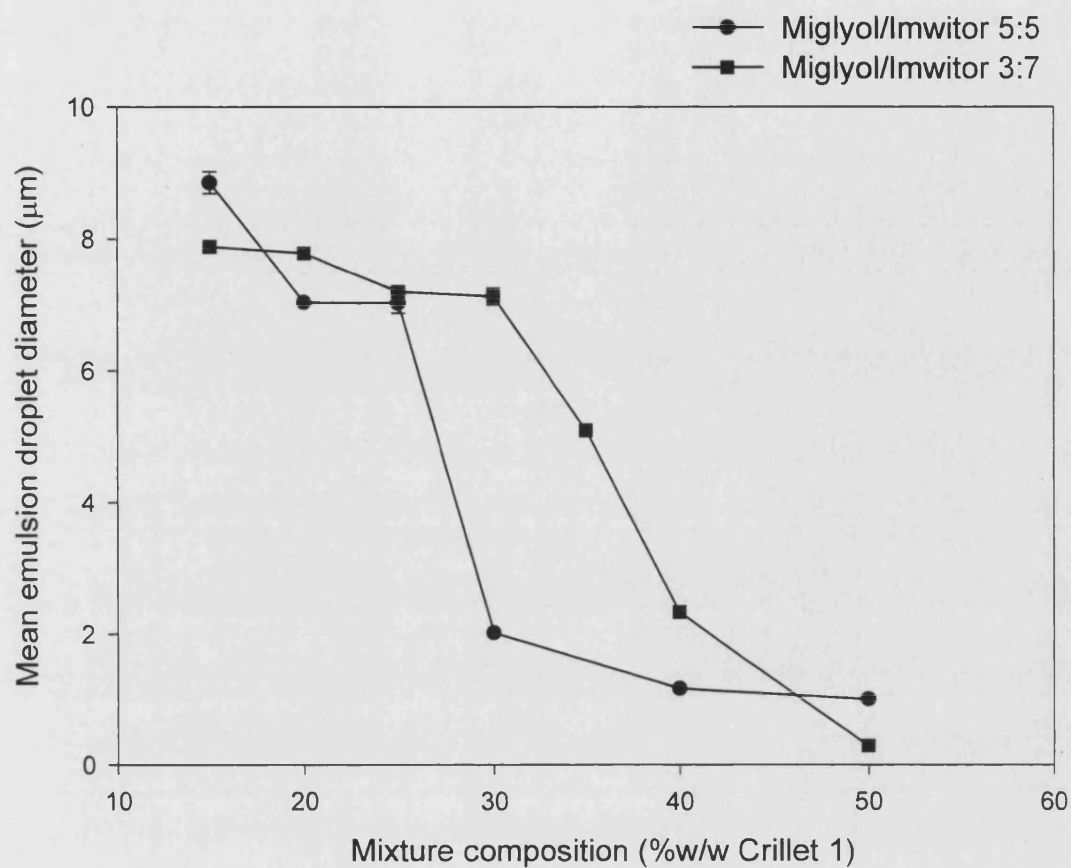
Three-dimensional graph and contour plot representations of MEDD data for the emulsification of Miglyol 812/Imwitor 988-Crillet 4 system as a function of surfactant concentration and ratio of Imwitor 988/Miglyol 812 in the oil blend.

of 30nm at cosurfactant-oil ratio of 60:40 and using approximately 35% w/w Crillet 4 i.e. 65(60/40)35. This ratio of oil blend was also found to be the optimum in a study by Kawakami et al. [31]. They found that the combination of MCG with MCT at the ratio of 6:4 in the case of Tween 80 (equivalent to Crillet 4) was effective to achieve maximum oil solubilization.

It is important to note here that the use of either Cremophor RH40 or EL has been able to produce SMEDDS with wider range of Miglyol 812/Imwitor blends than in the case of Crillet 4 (polysorbate 80). Hence Cremophor RH40 and EL are castor oil derivatives, these results suggest that the type and structure of the lipophilic moiety is of paramount importance to the self-emulsification process. Furthermore, the freedom of chain movement and orientation imparted by the removal of the sorbitan ring should aid in the ease of molecular interactions [6]. This was based on the improved self-emulsifying behaviour when Tagat TO (polyoxyethylene-(25)-glycerol trioleate, HLB 11.0) was used instead of Tween 85 (polyoxyethylene-(20)-sorbitan trioleate, HLB 11.0) with triglycerides oils (Miglyol 812).

These results have shown that Cremophor RH40 is a good surfactant for formulation of SMEDDS yet is not a special case. It is possible to obtain very fine dispersions with other non-ionic surfactants such as, Cremophor EL and polysorbate 80, provided right oil-cosurfactant ratio is used to optimise the performance of SMEDDS (see table 3.2.1).

Moreover, in an attempt to screen for further pre-microemulsion concentrate systems, new hydrophilic surfactants were investigated in this study. The emulsification performance of Miglyol 812/Imwitor 988-Crillet 1 system is presented in figure 3.2.13. In contrast with mixtures containing Crillet 4, this system has shown no potential for pre-microemulsion formulations hence it has produced dispersions with high MEDD values at all Miglyol 812/Imwitor ratios. This was also observed by Kawakami et al. [31] whereby Tween 80 (equivalent to Crillet 4) was able to solubilise 23% w/w of oil mixture composed of 1:1 DCPG and MCG vis-à-vis 1% in the case of Tween 20 (equivalent to Crillet 1). This might be due to the fact that Crillet 1 (Polyoxyethylene-(20)-sorbitan monolaurate) has relatively higher HLB value (16.7) than Crillet 4 (polyoxyethylene-(20)-sorbitan monooleate, HLB 15.0). This indicates that in order to obtain an equivalent optimum  $HLB_{mix}$  of Miglyol 812/Imwitor 988-



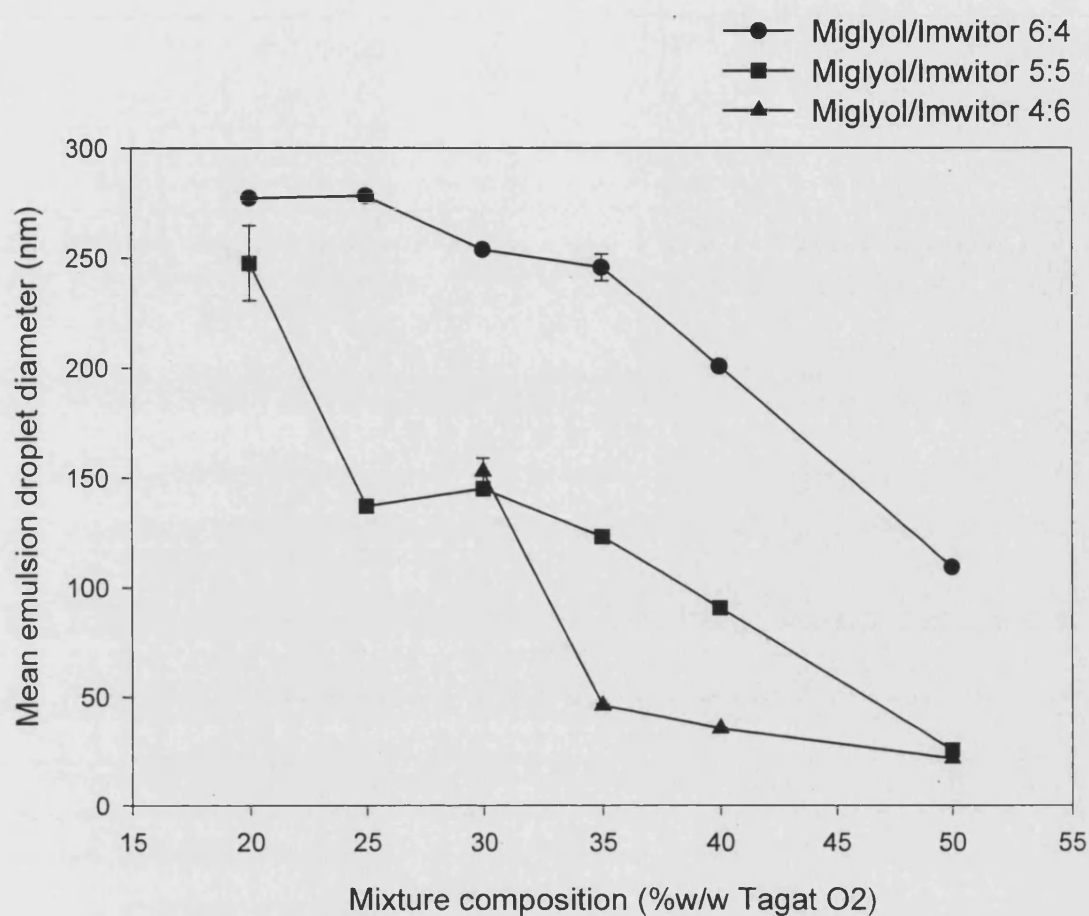
**Figure 3.2.13**

Emulsification performance as determined by droplet size measurement for self-emulsified systems containing Miglyol 812/Imwitor 988 ratios of 5:5 or 3:7 at increasing concentration of Crillet 1. Lipid formulations were emulsified in water at 37°C for 15 minutes. MEDD values were determined by laser diffraction method using the Mastersizer. Bars Represent Standard Errors (n = 3).

Crillet 4 system for producing microemulsion systems, Crillet 1 needs to be mixed with oil blends of high Miglyol 812 content for example; Miglyol 812/Imwitor 988 at 8:2 or may be 7:3 ratios. Yet, according to the ternary equilibrium phase diagram for Miglyol 812/Imwitor 988-Crillet 1 depicted in figure 3.2.4, these systems do not form stable isotropic one phase concentrates. Furthermore, it was surmised that the presence of oleic acid residues as the major constituent of the hydrophobic portion of the surfactant molecule were essential for mixtures with Miglyol 812 to exhibit good emulsification <sup>[6]</sup>.

The emulsification profiles of Miglyol 812/Imwitor 988 (6:4), (5:5) and (4:6) using Tagat O2 (polyoxyethylene-(20)-glyceryl monooleate, HLB =15) are presented in figure 3.2.14. As this figure depicts, microemulsion systems can be optimised using blends of Miglyol 812/Imwitor 988 at 4:6 ratio with Tagat O2 at concentration  $\geq 35\%$  w/w. Interestingly, this ratio of oil blend (4:6) is similar to the optimum ratio for SMEDDS in the case of Crillet 4. Yet at surfactant concentration  $< 30\%$  w/w, oil mixtures containing Crillet 4 appear to be more emulsifiable than Tagat O2. Yet, both surfactants at concentration  $\geq 35\%$  w/w produced fine dispersions with almost equivalent MEDD values (see table 3.2.2). Moreover, the emulsification performance of oil blends at ratios (5:5) and (6:4) using Tagat O2 (Figure 3.2.14) produced profiles comparable to the emulsification of same oil mixtures and in particular 5:5 ratio but containing Crillet 4 (Figure 3.2.11).

These results have demonstrated that the emulsification performance of oil systems containing either Tagat O2 or Crillet 4 have shown comparable trend with slight variations. This could be attributed to the fact that both surfactants have same HLB value (15) and more importantly similar chemical structure, yet with the lack of sorbitan ring in the case of Tagat O2. This suggests that the sorbitan nucleus structure in the case of polyoxyethylene monoalkyl esters is not the major factor to determine good self-emulsification with triglyceride oils. Similar observations were made by Wakerly <sup>[6]</sup>, whereby Crillet 11 (polyoxyethylene-(4)-sorbitan mono-laurate) gave a performance similar to alcohol ethoxylates of equivalent HLB. Further evidence was also demonstrated from studies with Atlox 1045A, an ethoxylated sorbitol (opened sorbitan ring) ester of mainly oleic acid (HLB 13.2). This surfactant exhibited good self-emulsification with Miglyol 812 and had an equivalent HLB value to Crillet 11 (same reference).



**Figure 3.2.14**

Emulsification profiles of self-emulsified systems containing Miglyol 812/Imwitor 988 ratios of 2:3, 1:1 or 3:2 at increasing concentration of Tagat O2. Lipid formulations were emulsified in water at 37°C for 15 minutes. MEDD values were measured by photon correlation spectrometer (PCS). Bars Represent Standard Errors (n = 3).

**Table 3.2.2: Comparison of Emulsification performance for microemulsion systems prepared by either Crillet 4 or Tagat O2 as determined by droplet size measurement.**

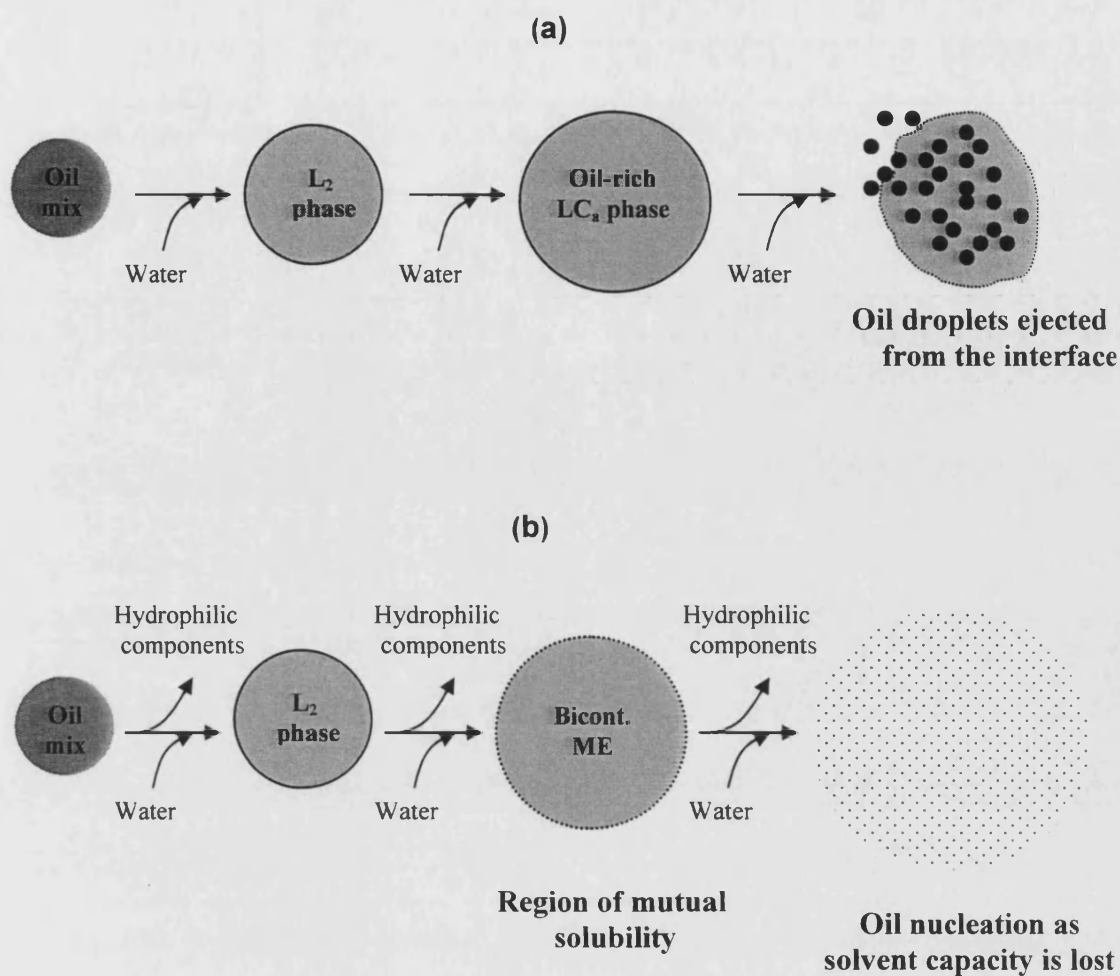
Formula	Miglyol/Imwitor (4:6)- Crillet 4	Miglyol/Imwitor (4:6)- Tagat O2
Surfactant Wt %	MEDD (nm)	
20	220.33±4.65	Off range
25	75.10±0.40	Off range
30	38.80±1.00	153.00±6.02
35	32.80±0.20	46.20±0.60
40	28.00±1.30	35.70±0.40
50	17.37±2.24	21.90±0.40

### 3.2.1.3. Mechanistics of Emulsification Process

General considerations of the putative mechanisms which have been proposed to explain spontaneous and self-emulsification processes have been discussed in detail in section 1.6. In practice, disruption of the oil-water interface is caused by penetration of water into the formulation or diffusion of hydrophilic components such as, cosolvents and hydrophilic surfactants away from the formulation into the aqueous phase (see figure 3.2.15). Both of these phenomena can be studied using equilibrium phase diagrams, which along side with droplet size analysis can allow the optimisation performance of Self-emulsified systems.

The precise mechanisms of emulsification remain the subject of speculation. Nonetheless, studies by Pouton <sup>[16]</sup> and Wakerly <sup>[6]</sup> using either Tween 85 or Tagat TO with Miglyol 812 have identified an empirical link between self-emulsification, liquid crystal formation, phase inversion temperature and enhanced water solubilization by oily systems.





**Figure 3.2.15**

Mechanistics of self-emulsification process; (a) Water-insoluble systems (eg. Type II SEDDS): penetration of water leads to the formation of liquid crystals at the oil-water interface which allow further penetration of water down aqueous channels, causing an increase in surface pressure and interfacial disruption which ensue in fine emulsion droplets to be exudated from the interface. (b) Systems containing a water-soluble component (Type III): 'diffusion and stranding' mechanism is attributed to the emulsification of these systems which can lead to very fine dispersions. As the solvent capacity is lost due to the diffusion of the hydrophilic component, oil and drug form a separate phase.

Nevertheless, for Miglyol 812/Imwitor 988-Tagat TO system (Section 3.1), we have shown that the enhancement of water solubilization ( $L_2$  phase) is of paramount importance to the mechanistics of emulsification. Furthermore, the enhanced water solubilization is linked to ease of emulsification and low droplet size <sup>[38]</sup>. This also appears to be true for type III systems investigated here which contain hydrophilic materials (surfactants with HLB >12 and/or water-soluble cosolvents) yet, this is further elaborated next.

For these systems where a hydrophilic surfactant or cosolvent is present rapid emulsification occurs by a mechanism which could be delineated as 'diffusion and stranding' (Figure 3.2.15). Diffusion of water into the oil phase converts it to w/o microemulsion ( $L_2$  phase) then there will be a significant migration of material which partitions and becomes diluted into the bulk aqueous phase <sup>[39]</sup>. This hydrophilic material dissolves the water-insoluble component into the aqueous phase as a solubilized system forming a bicontinuous microemulsion. On further diffusion, the concentration of hydrophilic component becomes too dilute to fully solubilise the oil and consequently the water insoluble oil separates as a second phase forming an oil droplet.

In order to obtain uniformly small oil droplets in water, the pre-concentrate oil mixture has to be either slightly lipophilic or hydrophilic depending on the hydrophilic-lipophilic balance (HLB) of the surfactant. Therefore, for systems containing lipophilic surfactants such as Tagat TO the injected drop has to be slightly lipophilic; i.e., the cosurfactant-to-oil ratio has to be slightly greater than that of the excess oil phase in equilibrium with bicontinuous microemulsion <sup>[19]</sup>. Weight ratio of oil-to-cosurfactant at 70/30 has been optimum for Tagat TO system, see table 3.2.3. On the other hand, in the case of hydrophilic surfactants such as Cremophor RH40, EL, and Crillet 4, oil blend (oil/cosurfactant) has to be slightly hydrophilic which means less weight ratio of oil (Miglyol 812) needs to be used (Table 3.2.3). Thus, for some optimal range of initial drop compositions, an emulsion of small oil droplets in water can be achieved.

**Table 3.2.3: Effect of surfactant hydrophilicity on the optimum weight ratio of oil in the oil-surfactant blend for various microemulsion systems. Surfactants with HLB values < 12 are considered to be lipophilic.**

Surfactant type	HLB of surfactant	Wt % of Miglyol in (Miglyol/Imwitor)
Tagat TO	11	70%
Cremophor EL	12-14	30-60%
Cremophor RH40	14-16	30-60%
Crillet 4	15	40%
Tagat O2	15	40%

In order to give insight into the mechanistic process of emulsification in type III systems, ternary equilibrium phase diagrams on dilution with water were constructed. Furthermore, emulsification profiles as determined by droplet size measurement were also analyzed in the view of equilibrium phase behaviour study. Miglyol 812/Imwitor 988-Cremophor RH40 system was selected for this phase study as an archetypal example of type III formulations which forms fine dispersions.

The triangular equilibrium phase behaviour diagram for Miglyol 812/Imwitor (5:5)-Cremophor RH40 system on dilution with water is depicted in figure 3.2.16. The oil blend of Miglyol 812/Imwitor 988 at ratio of 5:5 was first investigated here as it was found to be the optimum for self-micro-emulsification in the case of Cremophor RH40. As figure 3.2.16 displays, Miglyol 812/Imwitor 988 (5:5) blend is virtually immiscible with water as it forms two phase system ( $L_1+L_2$ ). On the other hand, Cremophor RH 40 as a hydrophilic surfactant with HLB value of 14-16 is soluble in water. Therefore, increasing the oil content in the formulation results, on aqueous dilution, in expansion of the  $L_1+L_2$  region and consequently, more surfactant is needed to homogenize the system into isotropic mixtures ( $L_2$  or  $L_1$ ).  $L_1+L_2$  region reached a maximum when the system contained 45-65% w/w oil and hence, minimum Cremophor RH40 concentration of 25% w/w was required to obtain clear mixtures close to the water apex in the ternary phase diagram, see figure 3.2.16.

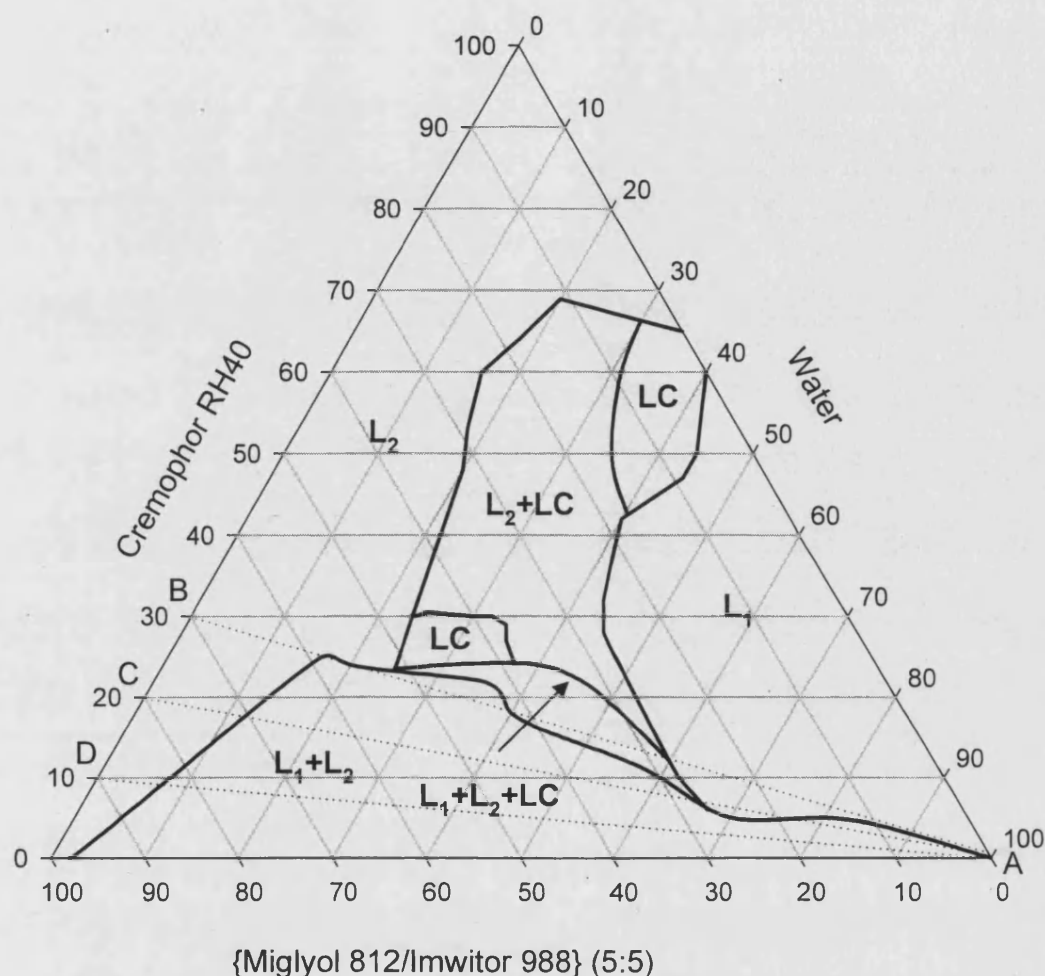


Figure 3.2.16

Triangular equilibrium phase diagram for the Miglyol 812/Imwitor 988 (5:5)-Cremophor RH40-water system at 25°C. Lines A-B, A-C and A-D represent the dilution of Miglyol 812/Imwitor 988 (5:5) blend and Cremophor RH40 mixtures at ratios 70/30, 80/20 or 90/10, respectively, with water. Aqueous-based liquids are designated  $L_1$ , oil-based liquids ( $L_2$ ) and liquid crystal phases “white” birefringence (LC). Turbid mixtures, which tended to separate on storage into a water-rich phase and an oil-rich phase, were designated ( $L_1+L_2$ ) or ( $L_1+L_2+LC$ ) when liquid crystalline material was clearly present.

Interestingly, this system at Cremophor RH40 ratio fraction in the oil mixture of >20 and on dilution with water between 20 and 50% w/w formed areas of various phases containing liquid crystalline material (LC,  $L_2$ +LC and  $L_1$ + $L_2$ +LC) which intercepted  $L_1$  and  $L_2$  regions (see footnote in figure 3.2.16 for phase description). Furthermore, an area of micellar solution ( $L_1$ ) was obtained on further dilution with water (>50% w/w). The ability of this system to solubilise the oil into micellar solution is attributable to the robust solubilizing capacity of Cremophor RH40 provided sufficient concentration is used. The LC phase and in particular the typical  $LC_a$  is generally associated with self-emulsification. The LC phase is viscous and exhibits white birefringence. In contrast, the transparent liquid crystalline dispersion phase, denoted  $LC_a$ , differs from the LC phase in that it displays low viscosity and multicolored birefringence. The nature of the  $LC_a$  is thought to consist of dispersion of lamellar liquid crystal in  $L_2$  phase [7, 8]. There has been no attempt in this phase study to differentiate between various types of LC phases, as their role in the mechanistics of emulsification for this system appears to be not crucial. Yet, the formation of LC phases after maximum solubilization of water ( $L_2$ ) of approximately 20% occurred, might have facilitated further penetration of water through the aqueous channels which resulted in further diffusion of the Cremophor RH40 away to the aqueous phase and consequently, the system became solubilized into micellar solution ( $L_1$ ).

The line (A-B) depicted in figure 3.2.16 represents the dilution of Miglyol 812/Imwitor 988 (5:5) blend and Cremophor RH40 mixtures at ratio of 70/30 with water. The dilution of the initial oil mixture ( $L_2$ ) with water would pass through  $L_2 \rightarrow L_1+L_2 \rightarrow L_1+L_2+LC_a \rightarrow L_1$  phases. Therefore, it is likely that the MEDD profile for the previous system (70(50/50)30) which is presented in figure 3.2.6 is considered to be the dilution of the micellar solution ( $L_1$  phase) with water to give a final composition which is close to the water apex in the triangular phase diagram. On the other hand, the emulsification of Miglyol 812/Imwitor 988 (5:5)-Cremophor RH40 at ratios of (90(50/50)10) depicted in figure 3.2.6 is considered to be the dilution of  $L_1+L_2$  phase (figure 3.2.16, line A-D). Therefore, it is anticipated for this system to produce high MEDD values. Nonetheless, increasing the surfactant ratio in the oil-surfactant pre-concentrate would, on aqueous dilution, shift the  $L_1+L_2$  phase towards the micellar solution region ( $L_1$ ) close to the water apex in the triangular phase

diagram (lines A-D, A-C, and A-B, Figure 3.2.16), and consequently fine dispersions are obtained. This is in agreement with the droplet size measurements depicted in figure 3.2.6, whereby MEDD is reduced by increasing the surfactant concentration in the oil system. Furthermore, as the line A-B depicted in figure 3.2.16 shows, Miglyol 812/Imwitor 988 (5:5)-Cremophor RH40 system at ratio of 70/30 is thought to be the optimum for micro-emulsification in this system, which also agrees with the contour representation of the MEDD depicted in figure 3.2.7.

In order to investigate the importance of triglyceride source (Miglyol 812) in the mechanistics of emulsification in type III systems, equilibrium phase behaviour for Imwitor 988-Crmophor RH40 system on dilution with water was studied and presented in figure 3.2.17. The ternary equilibrium phase diagram for Imwitor 988-Cremophor RH40-water system shows huge extension in the  $L_1+L_2$  region with comparison to the same system but containing Miglyol 812/Imwitor 988 at 1:1 ratio (Figure 3.2.16). For Imwitor 988-Cremophor RH40-water system, an optimum oil-to-surfactant ratio at 50/50 is required to transform two-phase mixtures into isotropic phases ( $L_2$  or  $L_1$ ) (line A-B, Figure 3.2.17) vis-à-vis 70/30 in the case of Miglyol 812/Imwitor (5:5)-Cremophor RH40 system (line A-B, Figure 3.2.16). On the other hand, the emulsification of Imwitor 988-Cremophor RH40 at ratio of 70/30 is considered to be the dilution of  $L_1+L_2$  phase (line A-C, Figure 3.2.17) and hence it is anticipated for this system to produce relatively high MEDD values. This indicates that in order to obtain pre-microemulsion concentrate in the case of Imwitor 988-Cremophor RH40 system more surfactant needs to be used which might raise certain toxicity issues. Therefore, including Miglyol 812 in the oil blend is important to obtain microemulsion systems in the case of type III lipid formulations. However, Miglyol 812 is required at optimum ratios to produce a pre-concentrate of oil-cosurfactant-surfactant with right  $HLB_{mix}$  for self-micro-emulsification.

Furthermore, in contrast to the extended areas which contained LC material in the case of Miglyol 812/Imwitor (5:5)-Cremophor RH40-water system, limited areas of LC phases were observed in the Imwitor 988-Cremophor RH40-water system (Figures 3.2.16 and 17, respectively). This might be attributed to the solvent capacity of Imwitor 988 in dissolving LC material due to high monoglyceride content and moreover, its ability as polar oil to depress the phase inversion temperature (PIT) of the system.

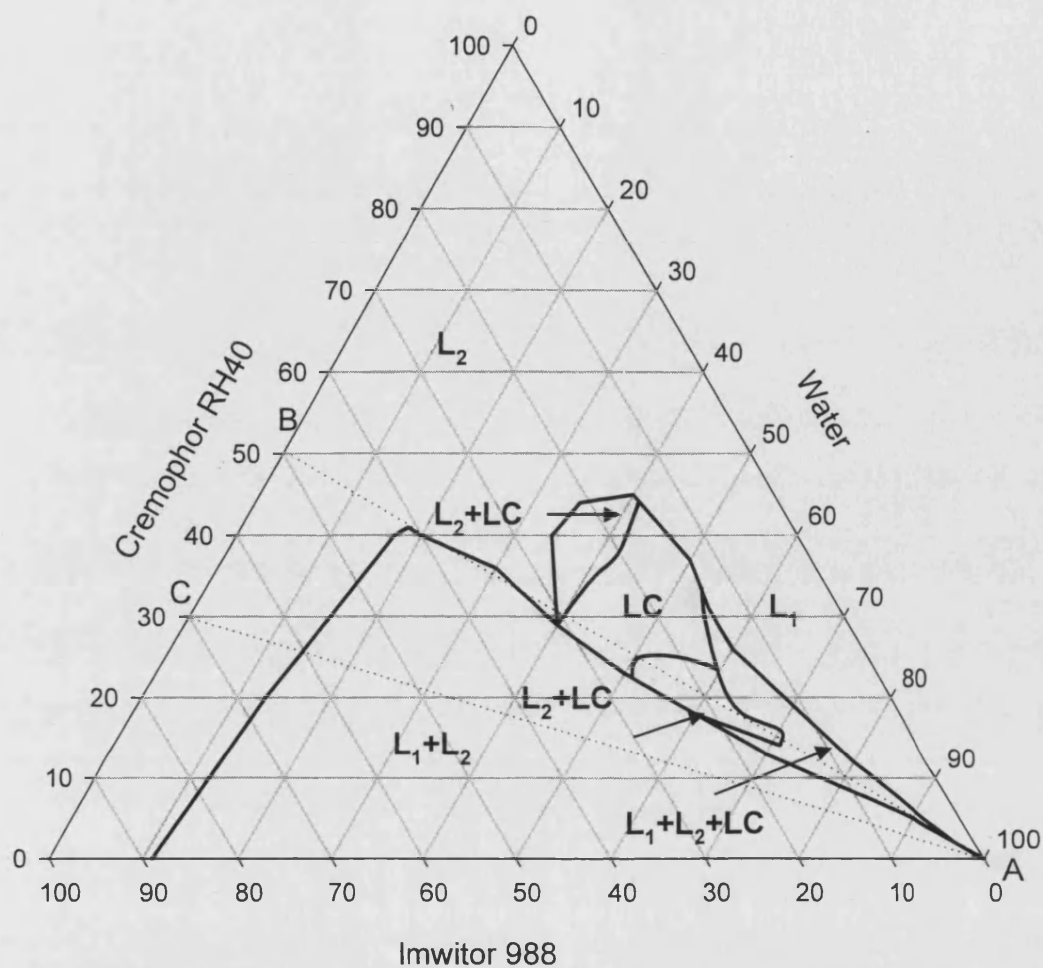


Figure 3.2.17

Triangular equilibrium phase diagram for the Imwitor 988-Cremophor RH40-water system at 25°C. Lines A-B and A-C represent the dilution of Imwitor 988/Cremophor RH40 binary mixtures at ratios 50/50 and 70/30, respectively, with water.

Generally, the emulsification performance of lipid systems which contain non-ionic surfactants are mostly affected by electrolytes in the aqueous phase, emulsification temperature and the inclusion of polar oils. These parameters cause depression in the PIT of the system by increasing the solubility of surfactant in oil and consequently phase separation occurs. In contrast to Miglyol 812/Imwitor 988-Tagat TO system which was thoroughly studied in the previous section, type III lipid systems described here appear not to be affected by the ionic strength of the aqueous phase. This is shown in our investigation next section of the effect of emulsification media on the performance of various lipid systems in the presence of a model drug. This might be attributed to the relatively high HLB values for these surfactants which are used in type III systems. The reduction, therefore, in the hydrophilicity of such surfactants incurred by the presence of electrolytes in the aqueous phase would not be sufficient to induce phase separation and thus dispersions with almost identical droplet size are obtained. In a similar study by Gao et al. <sup>[30]</sup> using a system composed of Captex 355<sup>®</sup> as an oil, Cremophor EL<sup>®</sup> as a surfactant, Transcutol<sup>®</sup> as a cosurfactant and saline they found that, microemulsions produced by adding 0.1 N HCL was of equivalence MEDD values to that produced by adding saline as an aqueous phase. Yet, further studies need to be carried out to investigate the effectiveness of these systems in vivo.

### 3.2.2. Concluding Remarks

The emergence of Neoral<sup>®</sup> by Novartis as a microemulsion system for drug delivery has generated the impetus amongst the researchers to investigate its merits as a replacement for the earlier 'Sandimmune' and moreover, to search for better alternatives. There have been many recent publications on the formulation, biopharmaceutics and pharmacokinetics of cyclosporin A formulated in a type IIIB lipid class system (Neoral<sup>®</sup>), yet not many of these reports have investigated the various parameters which influence the physicochemical characteristics of the resultant aqueous dispersion of the lipid vehicle.

The oil-cosurfactant ratio is one of these factors which affect the physicochemical characteristics of the resultant microemulsion systems designed for oral delivery. Oil blends of Miglyol 812 as an oil and Imwitor 988 as a cosurfactant were optimized for



microemulsion systems at ratios of 1:1 in the case of Cremophor RH40 or EL, and at 2:3 in the case of Crillet 4 or Tagat O2. Furthermore, Chemical structure of surfactants and their HLB values are amongst the important factors which affect microemulsion systems. For type III lipid systems to obtain small droplet size and fast dispersion rate, hydrophilic surfactants with HLB values between 13 and 15 were found to be the best. Furthermore, hydrophilic surfactants of castor oil derivative or with oleic acid residue were found to be the most effective for microemulsion systems.

Spontaneous micro-emulsification in type III lipid system was attributed to the “diffusion and stranding” theory whereby, diffusion of water into the oil mixture caused migration of the hydrophilic material away into the aqueous phase forming a bicontinuous microemulsion. Further migration of hydrophilic material due to further diffusion of water into the system caused the microemulsion to become supersaturated in oil, leading to nucleation oil droplets.

These microemulsion systems described here might have the potentials for pharmaceutical applications to improve the oral bioavailability of poorly-water soluble drugs yet, further in vivo investigations are required to establish the validity of such systems.

### 3.3. Effect of Physicochemical Properties of Emulsions Formed by Self-emulsifying Drug Delivery Systems (SEDDS) on the Solubilization State of Drug in Vitro.

#### 3.3.1. Results and Discussion

Absorption of drugs from the gastrointestinal (GI) tract is often dissolution rate-limited. A drug may be defined as 'poorly soluble' when its dissolution rate is so slow that dissolution takes longer than the transit time past its absorptive sites which results in poor bioavailability. There are many physical and physiological parameters important to dissolution which can be identified from the slightly modified form of Noyes-Whitney equation <sup>[40]</sup>:

$$DR = \frac{dX}{dt} = \frac{A \cdot D}{h} * \left\{ C_s - \frac{Xd}{V} \right\} \quad (\text{Eq. 1})$$

This equation shows that dissolution rate,  $DR$ , is a function of: the surface area of the exposed drug ( $A$ ), the diffusion coefficient of the drug ( $D$ ), the effective thickness of the boundary layer ( $h$ ), the saturation solubility of the drug under the local gastrointestinal conditions ( $C_s$ ), the amount of dissolved drug ( $X_d$ ) and  $V$  the volume of dissolution fluid. Many of these physicochemical and physiological factors which can have a great effect on the dissolution rate have been reviewed by Hörter et al <sup>[41]</sup>. Nevertheless, the aqueous solubility of a drug ( $S_0$ ) is a prime determinant of its dissolution rate and is usually less than  $100\mu\text{g/ml}$  <sup>[41]</sup> for 'poorly soluble' compounds which often present problematic dissolution to absorption.

The medium into which the drug must dissolve has a great influence on its solubility hence; suitable media which satisfactorily simulate the physiological conditions are needed. In the GI tract, solubility of drug is a function of aqueous solubility, crystallinity, drug lipophilicity,  $pK_a$  in relation to the pH profile of GI tract, solubilization by native surfactants (such as, bile salts and lecithin) and ingested food components.

Four suitable media have been suggested to simulate the composition of proximal GI tract<sup>[42]</sup>: SGF plus surfactant for fasted state stomach; long-life milk (3.5% fat) for fed state stomach; FaSSIF and FeSSIF for fasted and fed state small intestine, respectively (see, table 2.4 and 2.5 in chapter 2).

Furthermore, the effective permeability ( $P_{\text{eff}}$ ) is one of the physiological parameters that is crucial to drug dissolution and hence bioavailability. The transfer of most drugs across membranes occurs by passive diffusion from a region of higher concentration to one with lower concentration. Applying Fick's First Law to a membrane<sup>[43]</sup>, the absorption across the mucosal surface can be written as in Eq. (2)

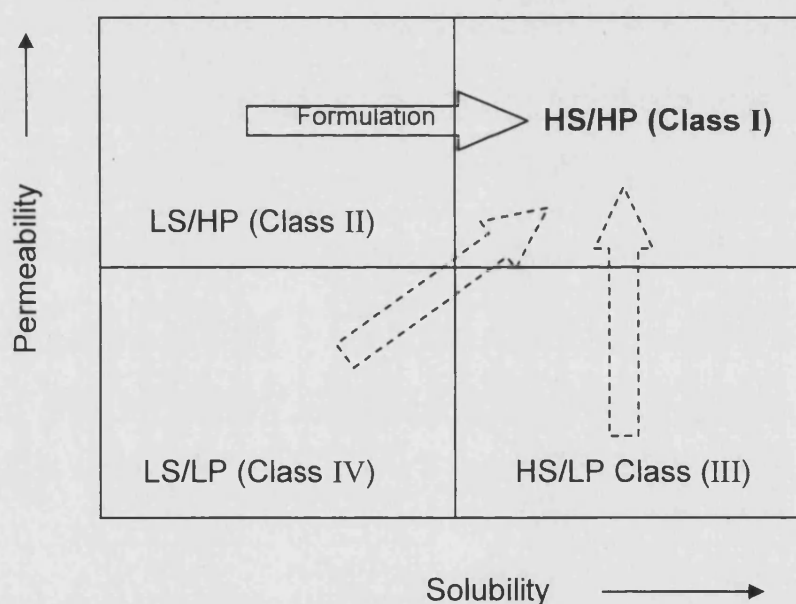
$$J_w = P_w \times C_w = \frac{dM}{dt} \times \frac{1}{A} \quad (\text{Eq. 2})$$

Where  $J_w$  is the mass transport across the gut wall,  $P_w$  can be assumed as the effective permeability,  $C_w$  is the concentration of the drug at the membrane and  $A$  is the surface area. According to Eq (2) permeability and solubility are the important parameters to determine the mass transport through a membrane. If the  $P_{\text{eff}}$  has a value of more than 2, complete absorption can be expected and hence the drug is classified as highly permeable. On the other hand, if the  $P_{\text{eff}}$  value of a drug is below 2, the drug is considered to be low membrane permeable as its absorption will be incomplete<sup>[44]</sup>. Highly permeable drugs are quickly absorbed and consequently concentrations in solution will remain lower, thus maintaining a maximal thrust for dissolution. Therefore, intestinal permeability to the drug can indirectly influence the dissolution rate<sup>[40]</sup>.

The Biopharmaceutics Classification System<sup>[44]</sup> which classifies drugs into four categories depending on inter-related functions of compound permeability and solubility (table 3.3.1), is useful for identifying candidate compounds for oral lipid-based formulations to improve bioavailability. According to this paradigm, drugs in class I have high permeability and high solubility hence, they are generally well absorbed (> 90%). Class II drugs are those with too low aqueous solubility, and high membrane permeability. Class III drugs have good solubility with membrane permeability too low for absorption to be complete. Class IV compounds have neither sufficient solubility nor permeability for complete absorption to occur.

**Table 3.1: Selecting Candidate drugs for oral delivery of lipid systems based on The Biopharmaceutics Classification System (BCS)** (Modified from Ref. 40)

BCS	Class I	Class II	Class III	Class IV
Physical and Physiological Parameters				
Aqueous Solubility	High	Low	High	Low
Membrane permeability	High	High	Low	Low
Potential Lipid-Based Emulsion System	w/o or o/w	o/w	w/o	o/w



**Figure 3.3.1**

Scheme showing the possible improvement in absorption for candidate compounds based on the biopharmaceutics classification system (BCS) by the use of lipid-based formulations [adapted from Ref. 45]. According to the BCS, Class I drugs have high permeability (HP) and high solubility (HS), Class II drugs; low solubility (LS) and high permeability (HP), Class III drugs; high solubility (HS) and low permeability (LP) and Class IV drugs; low solubility (LS) and low permeability (LP).

Oral bioavailability for selected compounds in every BCS category can possibly be enhanced via reformulation in oil vehicle. Yet, promoting apparent shifts in drug class from Class III and Class IV to class I by enhancement in intestinal permeability are not well characterised in terms of toxicity and are generally avoided <sup>[45]</sup>, dashed arrows in figure 3.3.1. In contrast, BCS Category II compounds which have low solubility (LS) and high permeability (LP), tend to demonstrate the most substantial enhancements in bioavailability when formulated in a solubilizing lipid excipient. Therefore, by the use of proper formulation strategies, it is possible to promote an apparent shift in drug properties from Class II to Class I (Figure 3.3.1).

In designing formulation strategies for enhancing the absorption and thus the bioavailability of 'poorly soluble' drugs, probing the solubility behaviour of these compounds in relation to the GI tract pH profile is crucial. As many drugs are weak electrolytes (acids or bases), the solubility is dependent on their ionization constant,  $K_a$  and the pH of the dissolution media. The pH of the gastrointestinal fluids widely varies with location in the GI tract; in the fasted stomach typical pH values are in the range of 1-2 while in the upper small intestine pH values lie between 5 and 6.5.

Poorly soluble weak acids with  $pK_a$  values less than 7.5 such as furosemide and indomethacin <sup>[46]</sup> ( $pK_a = 3.9$  and  $4.5$ , respectively) are predominantly un-ionised (insoluble) in the pre-prandial gastric fluids at pH 1 while are essentially ionised in the upper small intestine whereby dissolution occurs first. However, very weak acids with  $pK_a$  values more than 8 such as phenytoin, theophylline, or glutethemide are essentially un-ionised over the physiological pH range of the GI tract thus, they are very much equivalent in the gut to non-electrolytes. By contrast, weak bases with  $pK_a$  less than 5 like dapson, diazepam or chlorodiazepoxide will be predominantly ionised in the stomach and will not be highly soluble in the small intestine. Hence, after gastric emptying delivers these compounds to the small intestine, precipitation is likely to occur. Their absorption is likely to be dissolution rate-limited, analogous to biopharmaceutical properties of non-electrolytes <sup>[27]</sup>.

Therefore, crystalline hydrophobic weak electrolytes are often absorbed to an adequate extent because their  $pK_a$  (s) allow them to exist predominantly as ionised species in the lumen of the gut. Hence, the drug is absorbed as the lipid-soluble un-

ionised species but the ionised species represents a reservoir of drug from which the un-ionised species is immediately available <sup>[47]</sup>. When the drug is a non-electrolyte or an electrolyte with  $pK_a$  (s) which are unfavourable for dissolution in the gut i.e. ( $pK_a > 8$  for weak acids and less than 5 in the case of basic compounds), assuming sufficient solvent capacity to allow for a unit dose formulation is achievable, reformulation using different types of oil systems (Type I, II or III) can improve absorption. These systems can provide a reservoir of drug dissolved in either lipid or micellar solution and hence slow dissolution of the unionised species is avoided.

Another key issue in the formulation design of lipid systems is to maintain solubility of the drug throughout its passage through the GI tract. Crystallization of the drug due to loss of solvent capacity of oil formulation on dilution in the lumen of the gut depends on the hydrophilicity of oil system;  $\log P$  of the drug and the solubilization capacity of native surfactants (bile salt-lecithin mixed micelles) to maintain the drug in solution during digestion. Many reports <sup>[40, 41 and 43]</sup> have dealt with the influence of drug physical features and many physiological parameters on the solubilization behaviour of poorly soluble drugs in the GI tract. Yet, the implementation of these studies in the formulation design of lipid systems, as an important approach to improve absorption, needs further focus in order for this technology to see widespread application within the pharmaceutical industry.

We are, however, investigating how the physicochemical properties of emulsions formed by various types of lipid systems can influence drug solubilization in an attempt to investigate the fate of dissolved drug after dispersion. Parameters in the oil pre-concentrate which can affect the solvent capacity of the resultant dispersion and thus lead to precipitation of the drug such as, oil-cosurfactant ratio, type of surfactant used in the system and the inclusion of water soluble co-solvents were studied. Furthermore, physicochemical properties such as droplet size of the resultant emulsions, ionic strength of the emulsification media and the solubilization capacity of bile salt-lecithin mixed micelles in relation to keeping the drug in solution and avoid crystallization were also investigated. Dimethyl Yellow (DMY) was used as a model 'poorly soluble' weak base ( $\log P$  of 4.52,  $pK_a$  of 3.226 and aqueous solubility ( $S_0$ ), = 1.33  $\mu\text{g/ml}$ ). Different types of self-emulsifying systems (type II, IIIA and IIIB) were used to probe the influence of the various physicochemical properties of the resultant dispersions on the fate of dissolved drug.

### 3.3.1.1. Solubility of Dimethyl Yellow (DMY) in the Lipid Matrix

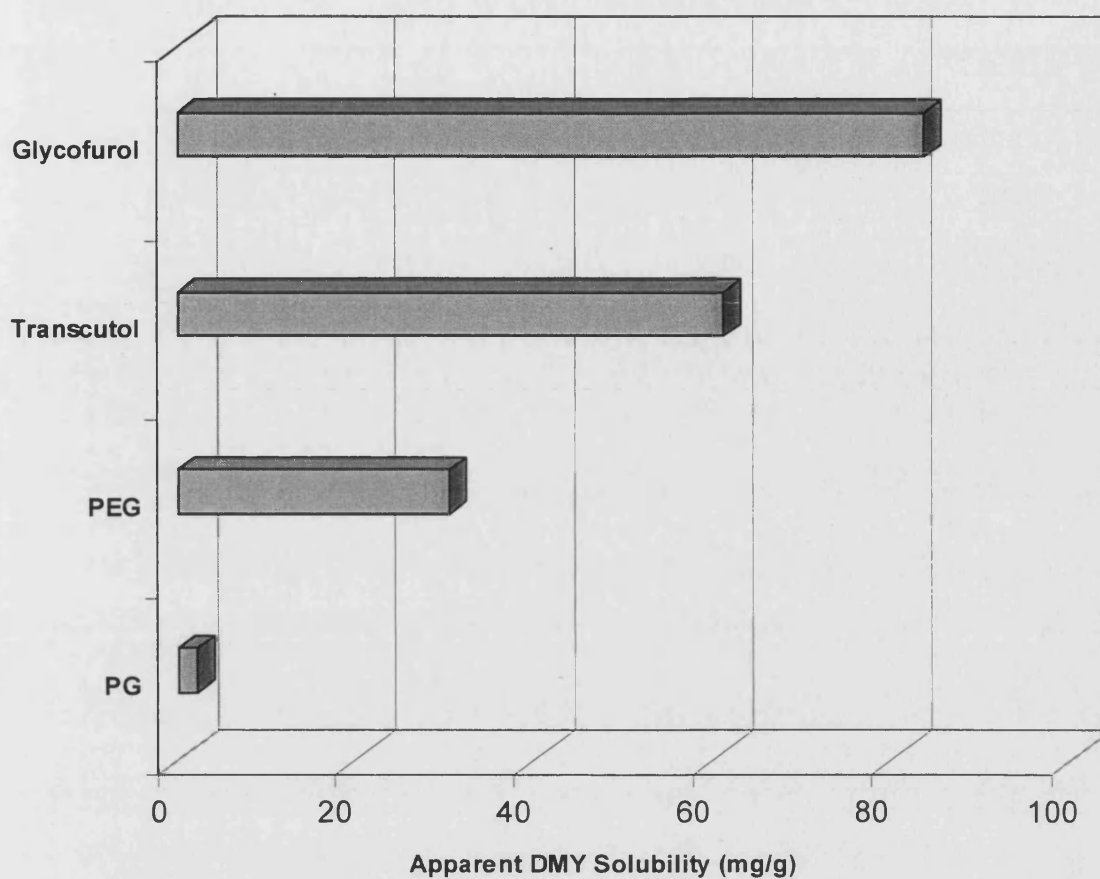
One commonly used approach to improve absorption is to solubilise the drug in a water-soluble cosolvent, such as polyethylene glycol which may have sufficient solvent capacity for drug administration as a unit dose. Yet, it is likely that the drug will crystallize on dilution of the cosolvent in the lumen of the gut. Unlike water-miscible cosolvent systems, lipids due to their immiscibility with water, can maintain poorly soluble drugs in solution. However, when oil systems can not provide sufficient solvent capacity for the drug in the unit dosage form, water-miscible cosolvents can be included in the pre-concentrate formulation. Therefore, verification of drug solubilization in the excipient matrix with a suitable assay for the drug is a crucial pre-formulation step that should be undertaken to assess the viability of any lipid system.

The solubility of DMY in various water-miscible cosolvents and also in different types of lipid-based formulations is depicted in figures 3.3.2 and 3.3.3, respectively. Maximum solubilization of DMY was obtained in Glycofurol system followed by Transcutol, see figure 3.3.2. An approximately 2.5 fold decrease in the DMY solubility was observed in Polyethylene glycol 400 system vis-à-vis Glycofurol. The solubility of DMY, on the other hand, was the lowest in the case of Propylene glycol system.

Considering the cosolvent solubilization power ( $\sigma$ ) concept in the log-linear model proposed by Yalkowsky and coworkers <sup>[48, 49, 50]</sup>, we can retrospectively work out  $\sigma$  for DMY in the various cosolvent systems which were used in this study. The log-linear model describes an exponential increase in the solubility of non-polar drugs with a linear increase in cosolvent concentration. This is presented in the following equation:

$$\text{Log } S_{\text{tot}} = \text{log } S_w + \sigma \times f_c \quad (\text{Eq. 3})$$

where  $S_{\text{tot}}$  is the total solubility of drug in the cosolvent-water mixture,  $S_w$  is its water solubility,  $\sigma$  is the cosolvent solubilization power for the particular cosolvent-solute system and  $f_c$  is the volume fraction of cosolvent in the aqueous mixture.



**Figure 3.3.2**

Apparent solubility of DMY in various cosolvent systems, the drug was added in excess to these systems and left to equilibrate at 25°C for 24h.



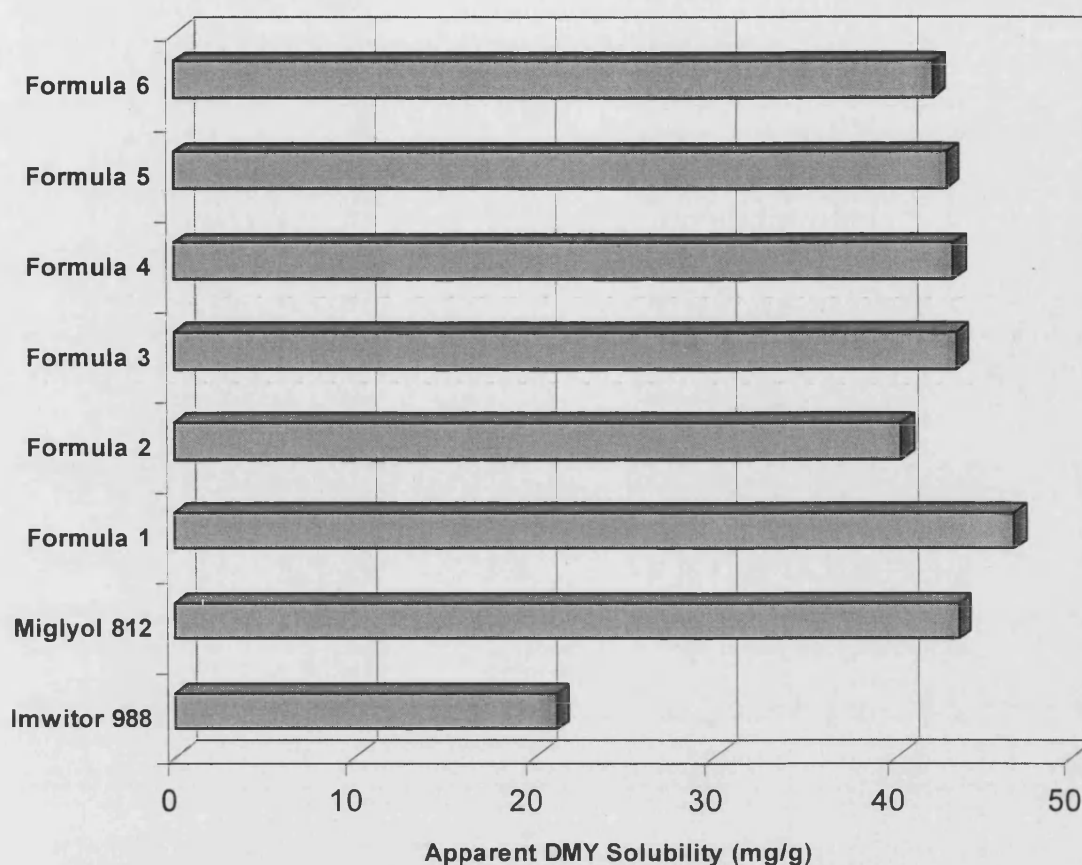
For this study  $\sigma$  was obtained from the  $\log (S_{\text{tot}} / S_{\text{w}})$  considering  $f_c = 1$  as no water was included in the cosolvent system. Table 3.3.2 lists the cosolvent solubilization power ( $\sigma$ ) obtained from Eq 3 and the partition coefficient ( $\log P$ ) of the considered cosolvent systems. The  $\log P$  values were obtained by means of the ClogP software package.

The  $\log P$  denotes the polarity of the cosolvent system. As table 3.3.2 illustrates, the higher the  $\log P$  of the cosolvent, the lower the polarity and hence the higher the solubilization power. The following order is observed:  $\log P_{\text{Glycofurol}} > \log P_{\text{Transcutol}} > \log P_{\text{PEG 400}} > \log P_{\text{PG}}$ . The least polar cosolvent, Glycofurol, has the maximum solubilization power for DMY ( $\sigma = 4.8$ ), and the highest polar cosolvent, PG, has the lowest value ( $\sigma = 3.19$ ). This is expected since less polar cosolvents can reduce self-association than polar cosolvents as they have better affinity for the non-polar compounds. It is worth noting here that, Polyethylene glycol 400 has higher  $\sigma$  value than Propylene glycol albeit they both have close  $\log P$  values. This might be due to the large area of interaction with the DMY in the case of PEG 400 due to the relatively high number of polyoxyethylene residues (an average of 9 residues per molecule).

**Table 3.3.2: The effect of cosolvent polarity on the cosolvent solubilization power ( $\sigma$ ) for DMY in PG, PEG 400, Transcutol and Glycofurol (sorted by increasing  $\log P$ ).**

Cosolvent System	Cosolvent $\log P$	Solubilisation power ( $\sigma$ )	Solubility of DMY mg/g
Propylene glycol	-0.92	3.19	2.06±0.002
Polyethylene glycol 400	-0.88	4.36	30.46±0.42
Transcutol	-0.15	4.66	60.99±1.54
Glycofurol	-0.04	4.80	83.53±1.69

Figure 3.3.3 displays the solubility of DMY in the different types of lipid systems (Type I, II, IIIA and IIIB). DMY solubility of  $\approx 40\text{mg/g}$  was obtained in almost all lipid systems investigated except for Imwitor 988 which measured  $20\text{mg/g}$ .



**Figure 3.3.3**

Apparent solubility of DMY in various Lipid systems; Formula 1 is composed of Miglyol 812/Tagat TO at ratio of (70/30); Formula 2 = Miglyol 812/Imwitor 988-Tagat TO 70(50/50)30; Formula 3 = Miglyol 812/Imwitor 988-Cremophor RH40 70(60/40)30; Formula 4 = Miglyol 812/Imwitor 988-Cremophor EL 70(70/30)30; Formula 5 = Miglyol 812/Imwitor 988-Crillet 4 70(70/30)30; Formula 6 = Imwitor 988/Cremophor RH40-PEG 400 30/30/40. According to Pouton's <sup>[5]</sup> classification; Formula 1 and 2 represent Type II class systems, Formula 3, 4 and 5 Type IIIA, and Formula 6 Type IIIB lipid system. The drug was added in excess to these systems and left to equilibrate at 25°C for 24h.

Furthermore, identical solubilization power of 4.5 was found for these lipid systems, yet, with lower value in the case of Imwitor 988 ( $\sigma = 4.2$ ) as it is a more polar oil due to the monoglyceride content. On the other hand, the inclusion of PEG 400 in the oil mixture to formulate Type IIIB system (Figure 3.3.3, formula 6) did not, however, enhance the solubility of DMY as PEG 400 has a relatively low solubilization power. If a high DMY solubility is required, cosolvents with high solubilization power such as, Glycofurol and Transcutol can be used.

### **3.3.1.2. Physicochemical Parameters Influencing the Solvency of Dispersions Formed by Various Lipid-Based Formulations**

#### *Hydrophilicity of the lipid-Vehicle: Oil Formulations Vs Cosolvent Systems*

Studies generally, in the field of lipid-based formulations, focus on investigating the effect of drug on the physicochemical characteristics of the resultant dispersions in particular, emulsion droplet size as it is crucial for the enhanced bioavailability. Also, equilibrium phase behaviour is being studied in the presence of drug, especially if the drug has surface activity, in order to establish an empirical link between these effects incurred by the inclusion of a particular drug and emulsification. Nonetheless, there have not been many reports which outline how the physicochemical properties of emulsions formed by SEDDS can influence the fate of dissolved drug after dispersion. We are investigating the effect of solvent capacity of these dispersions on the solubilization behaviour of the drug and how factors such as, oil-cosurfactant ratio, type of surfactant and the inclusion of various cosolvents can influence this behaviour in an attempt to circumvent the precipitation of drug.

Photos presented in figures 3.3.4 and 3.3.5 outline the effect of various types of either lipid-based formulations or cosolvent systems on the precipitation of drug after dispersion of the formulation. Figure 3.3.4 shows the aqueous dispersions of oil formulations in the presence of approximately 40mg/g DMY representing Type II lipid-class system (bottles A & B), and Type IIIA (bottles C, D & E). In Type II lipid systems, the pre-concentrate mixture, which is composed of water insoluble materials, was selected to form either a self-emulsified dispersion of particle size more than 250nm or a microemulsion, bottles A and B, respectively. On the other

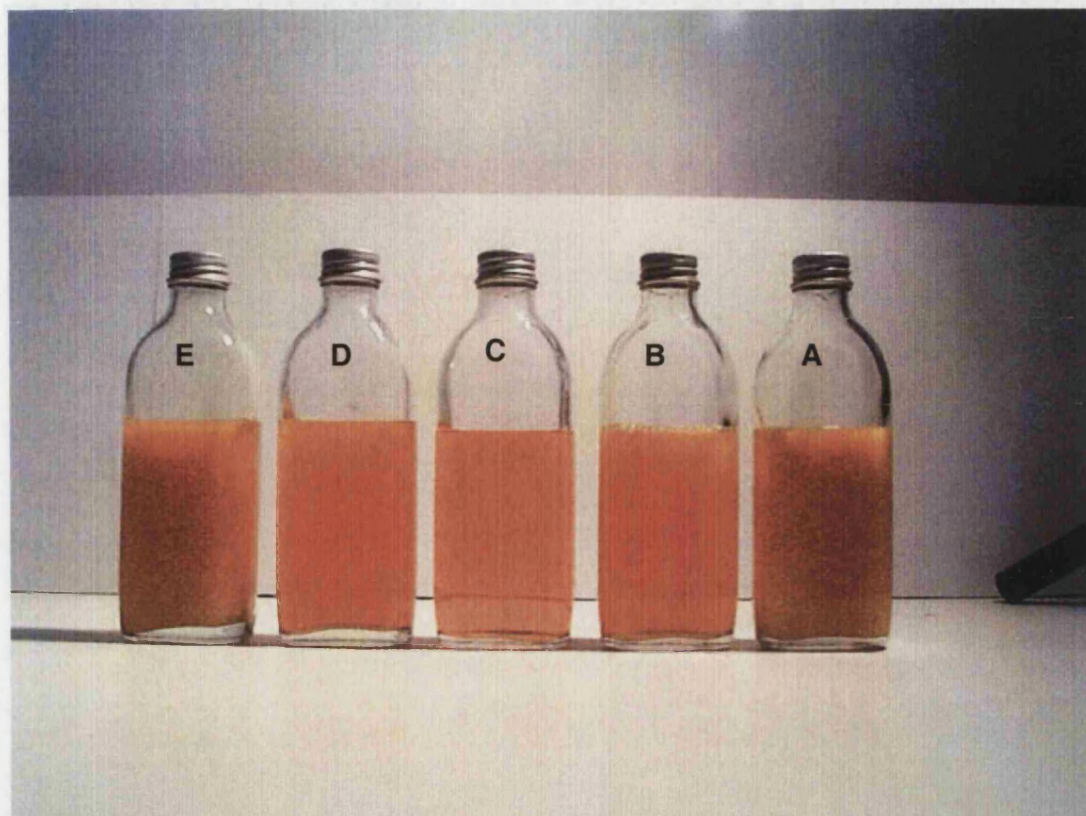


Figure 3.3.4

Photograph of the effect of various lipid systems (Type II and Type IIIA) on the solubilization of DMY after dispersion of the formulation. Bottles A to E represent dispersions of formulations from 1 to 5 denoted in figure 3.3.3. 1g of each formulation containing approximately 40mg DMY was allowed to emulsify in 100ml water at 37°C for 15 minutes. Dispersions were assessed visually and analytically for detecting any drug precipitation due to loss of solvent capacity.



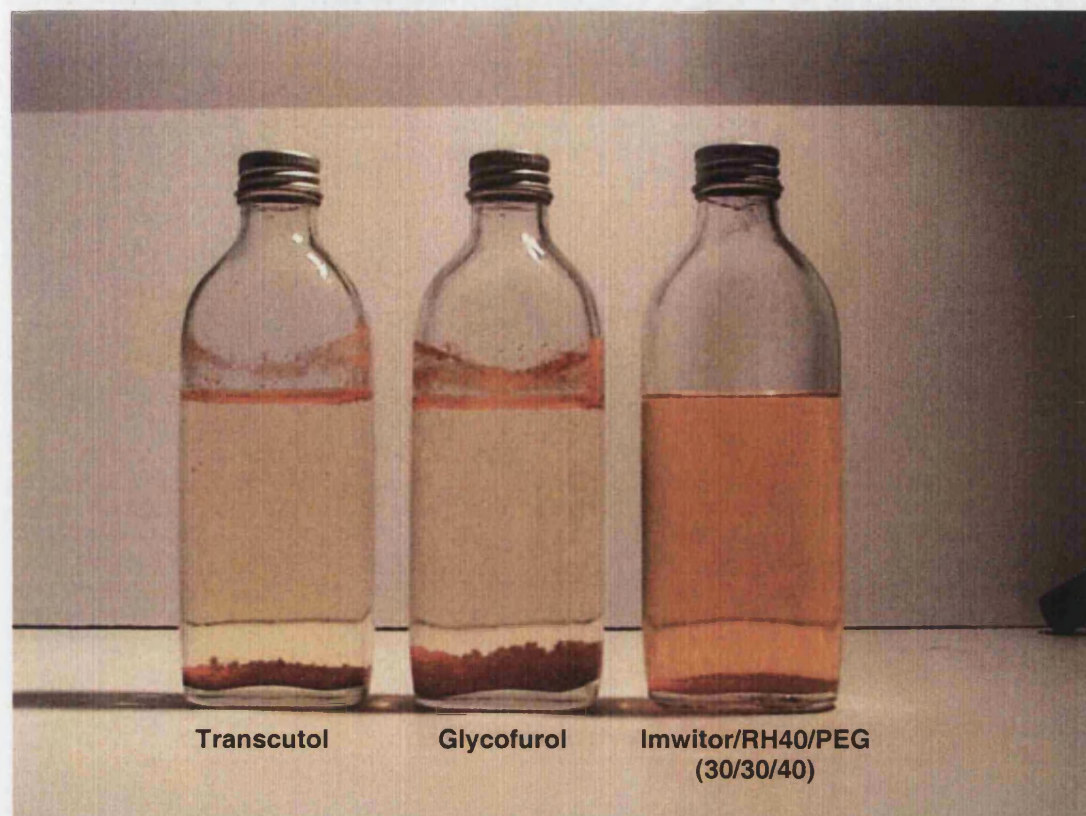


Figure 3.3.5

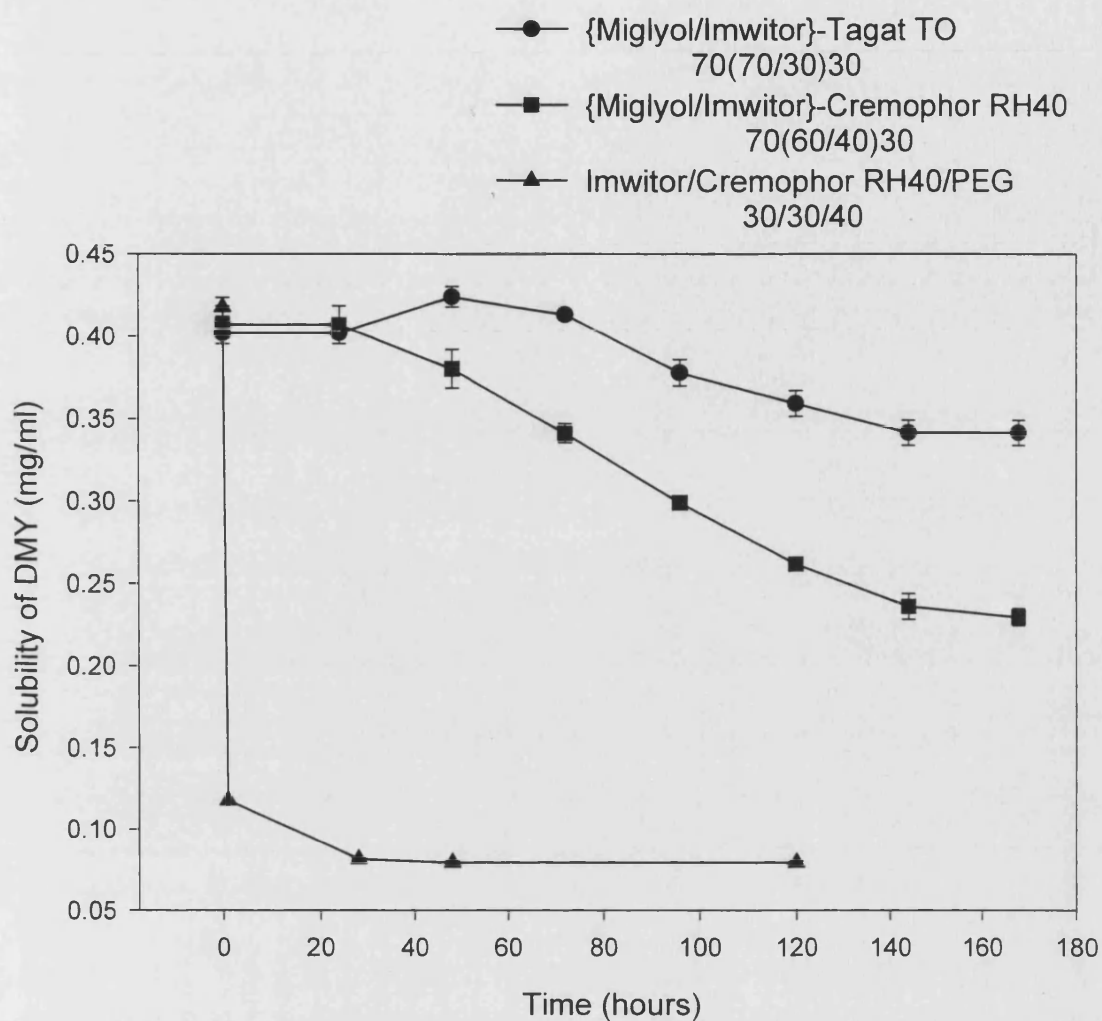
Photograph of the effect of either Type IIIB lipid class formulation (Imwitor 988/Cremophor RH40-PEG 400) or water-miscible cosolvent based systems (Glycofurol and Transcutol) on the solubilization of DMY after dispersion of the formulation. 1g of each formulation was emulsified in the presence of DMY in 100ml water at 37°C for 15 minutes.

hand, various types of hydrophilic surfactants Cremophor RH40, EL or Criliet 4 were included in Type IIIA systems, bottles, C, D or E, respectively. Visual assessment of dispersions depicted in figure 3.3.4 did not reveal any crystallization of DMY in the first 24 hours. Nonetheless, dispersions which formed fine emulsions (bottles B & C) were subjected to further analytical assessment for several days in order to study the solubilization behaviour of DMY with time in these systems.

Furthermore, an instant crystallization of DMY occurred from the emulsification of either Imwitor 988/RH40-PEG 400 (30/30/40) system, an archetypal example of Type IIIB lipid formulations, or cosolvent-based systems (Glycofurol and Transcutol), see photo in figure 3.3.5). Type IIIB formulations generally produce the finest dispersions because of their high content of water-soluble solubilizing agents, viz. they contain more than 60% hydrophilic surfactant and/or water-miscible cosolvents <sup>[5]</sup>. Hence, the water soluble components tend to diffuse away from the oil during dispersion, and become dissolved in the aqueous phase. The result of this separation, which may be the driving force for emulsification by ‘diffusion and stranding’, is likely to be loss of solvent capacity <sup>[5]</sup>. As a consequence, the drug is partially precipitated when the formulation disperses. This was also evident from the water-miscible cosolvent systems Glycofurol and Transcutol whereby, spontaneous crystallization of DMY occurred after dilution in the aqueous phase (figure 3.3.5).

The extent of precipitation will depend on hydrophilicity of the lipid system, the particle size of the resultant dispersion, the contribution of the hydrophilic material (hydrophilic surfactant and/or cosolvent) to the solubilization of the drug within the formulation and the log P of the drug.

Figure 3.3.6 displays the effect of hydrophilicity of the lipid matrix, as controlled by type of excipients included in the system, on the solubilization behaviour of DMY. The {Miglyol 812/Imwitor 988}-Tagat TO at ratio of (70(70/30)30) is a pre-microemulsion Type II lipid mixture. This type of formulation is composed of water insoluble materials i.e. a hydrophobic system. Hence, it is expected to retain its solvent capacity for the drug after dispersion. It is evident from the emulsification of this system in the presence of DMY that the solvency of the resultant dispersion was able to keep the drug in super-saturated state for up to 3 days after the emulsification event. However, at equilibrium which can take up to five days, only 6mg of DMY out



**Figure 3.3.6**

Effect of hydrophilicity of the oil vehicle on the solubilization profiles with time for DMY. Lipid formulations representing Type II, IIIA and IIIB and containing around 40mg/g DMY were allowed to emulsify (1g) in 100ml of water at 37°C for 15 minutes. Dispersions were kept for various periods of time for probing drug precipitation.

of an initial dose of approximately 40mg came out of solution. This might be due to the fact that this system forms fine dispersion on emulsification so oil droplets which contain the dissolved drug will be highly exposed to the aqueous phase due to large surface area of contact. Therefore, the system can not reduce water's ability to "squeeze out" the drug from the fine oil droplets and consequently drug crystallization might occur. This process, however, might take long time to reach equilibrium as it was demonstrated. On the other hand, the self-emulsified type II class lipid-system which forms dispersion with particle size of > 250 nm such as Miglyol 812/Tagat TO (bottle A, figure 3.3.4) had shown no indication for DMY crystallization. This might be attributable to the relatively large droplet size whereby, DMY will be sequestered within the oil phase. Hence, there will be virtually minimal interaction between the non-polar compound and the water's hydrogen network which will reduce the ability of water to induce crystallization due to self-association.

On the other hand, for Type IIIA lipid system, {Miglyol 812/Imwitor 988}-Cremophor RH40 at ratio of 70(60/40)30, DMY was able to remain in a super-saturated state up to 24 h after emulsification (figure 3.3.6). The crystallization of DMY from this system was also found to take up to 5 days to reach equilibrium. Unlike the limited crystallization which occurred at equilibrium from the self-micro-emulsified type II lipid-system (figure 3.3.6), approximately 42% of the initial DMY dose (40mg) precipitated out from the Type IIIA system. This might be due to two factors, one of which is the amount of DMY which the hydrophilic surfactant (Cremophor RH40) was contributing to its solubilization within the formulation.

The amount of solubilized DMY within the pre-concentrate mixture which is contributed by the hydrophilic surfactant can be calculated using the following equation:

$$S_{\text{total}} = F_A \times S_A + F_B \times S_B + F_C \times S_C \quad (\text{Eq. 4})$$

where  $S_{\text{total}}$  is the total solubility of DMY in the lipid mixture,  $F_A$ ,  $F_B$  and  $F_C$ ; mole fraction of each lipid excipient in the oil system, and  $S_A$ ,  $S_B$  and  $S_C$ ; the respective DMY solubility in each constituent.



In the case of {Miglyol 812/Imwitor 988}-Cremophor RH40 system at ratio of 70(60/40)30, an amount of approximately 19mg of DMY is solubilized within 1g of the oil mixture due to the contribution of Cremophor RH40. On emulsification of 1g formulation containing around 40mg DMY in 100ml of water, Cremophor RH40 which has a concentration of 30% w/w in the pre-concentrate, parts from the oil during dispersion and becomes diluted in the aqueous phase making a final concentration of 0.3% w/v. By applying the linear regression equation for Cremophor RH40 system presented in figure 3.3.7 which relates the increase of DMY solubility in Cremophor RH40-water system as a function of surfactant concentration, we can work out the amount of DMY that the surfactant can maintain in solution after the dispersion of the formulation. The dissolved surfactant in the aqueous phase after dispersion can maintain an amount of around 5mg of DMY in solution out of the 19mg which Cremophor RH40 is responsible for in the initial pre-concentrate dose. Therefore, approximately 14mg of DMY ( $\approx 35\%$  of initial dose) will tend to come out of solution at equilibrium as a result of diffusion of the hydrophilic surfactant into the aqueous phase after dispersion. Another important factor which might further initiate crystallization of DMY from Type IIIA lipid system is the large surface available for contact with water due to the formation of very fine oil particles after dispersion.

In the case of the very hydrophilic Type IIIB lipid system, the risk of precipitation is greater as the formulation contains a higher proportion of hydrophilic components (hydrophilic surfactants and/or water-soluble cosolvents). This is demonstrated by the instant crystallization of DMY from the Imwitor 988/Cremophor RH40-PEG 400 Type IIIB lipid system depicted in figure 3.3.6. Almost 70% of an initial DMY dose of  $\approx 40\text{mg}$  came out of solution in the first hour after the dispersion of the formulation. The amount of DMY that is dissolved within the lipid mixture due to the contribution of Cremophor RH40 or PEG 400 is around 23 or 12mg, respectively. This means that the hydrophilic components in the Imwitor 988/Cremophor RH40-PEG 400 system are responsible for solubilizing approximately 35mg of DMY in the lipid matrix. Cremophor RH40 and PEG 400 can keep up to only 5mg of DMY in solution after the dispersion of the formulation as they tend to lose their solvent capacity on dilution with water. Therefore, 30mg of DMY, which is almost equivalent to 75% of DMY initial dose, is expected to come out of solution after the

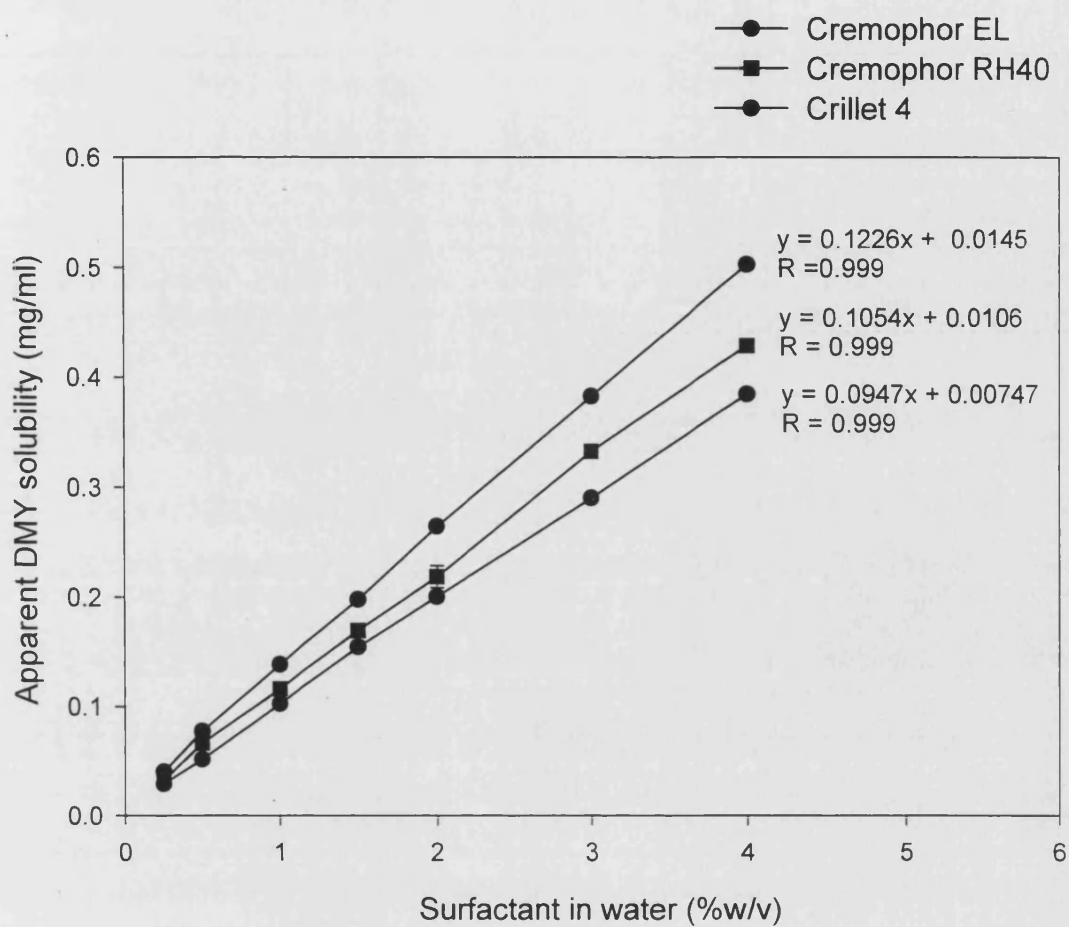


Figure 3.3.7

Plots of solubility of DMY in various surfactant-water mixtures as a function of surfactant concentration.

emulsification. This is, however, in full agreement with the solubilization profile of DMY from this system which is depicted in figure 3.3.6.

The partition coefficient ( $\log P$ ) of the drug is an important physicochemical factor which influences the formulation design for lipid systems. Generally, drugs with  $\log P$  values  $< 2$  are the most difficult drugs as they have limited solubility in both water and lipid. Therefore, it is unlikely that lipid formulation will be of value for such compounds <sup>[5]</sup>.

On the other hand, more hydrophobic drugs may have good permeability to lipid membranes but dissolve very slowly in the lumen of the gut. Hydrophobic drugs with intermediate  $\log P$  [2-4] may benefit the most from the formulation design of lipid-based systems. Hydrophilic surfactants and/or water-soluble cosolvents can be blended with the lipid systems to increase the solvent capacity of the formulation for molecules with intermediate  $\log P$ . Yet, the choice between the different types of lipid-based formulations (Type I, II or III) has to be made considering the risk of drug precipitation and also the desirability of rapid absorption. Furthermore, drugs with  $\log P > 2$  are likely to be solubilized by bile salt micelles during digestion which in effect might prevent the drug from precipitation in the gut.

Drugs with  $\log P > 5$  can also benefit from the reformulation with lipid systems yet digestion by lipolysis will be crucial for the absorption of these drugs. The natural process of digestion offers the possibility that highly lipophilic drugs with  $\log P$  values greater than 5 and triglyceride solubility of at least 50mg/ml are preferentially transported via lymphatic route <sup>[51, 52]</sup>. Lymphatic transport may be enhanced by lipid-based formulations yet the enhancement depends on the nature of the vehicle. According to a study by Porter et al <sup>[45]</sup>, the rate and extent of lymphatic transport of a highly lipophilic drug was of the following rank order; micellar  $>$  emulsion  $>$  lipid solution.

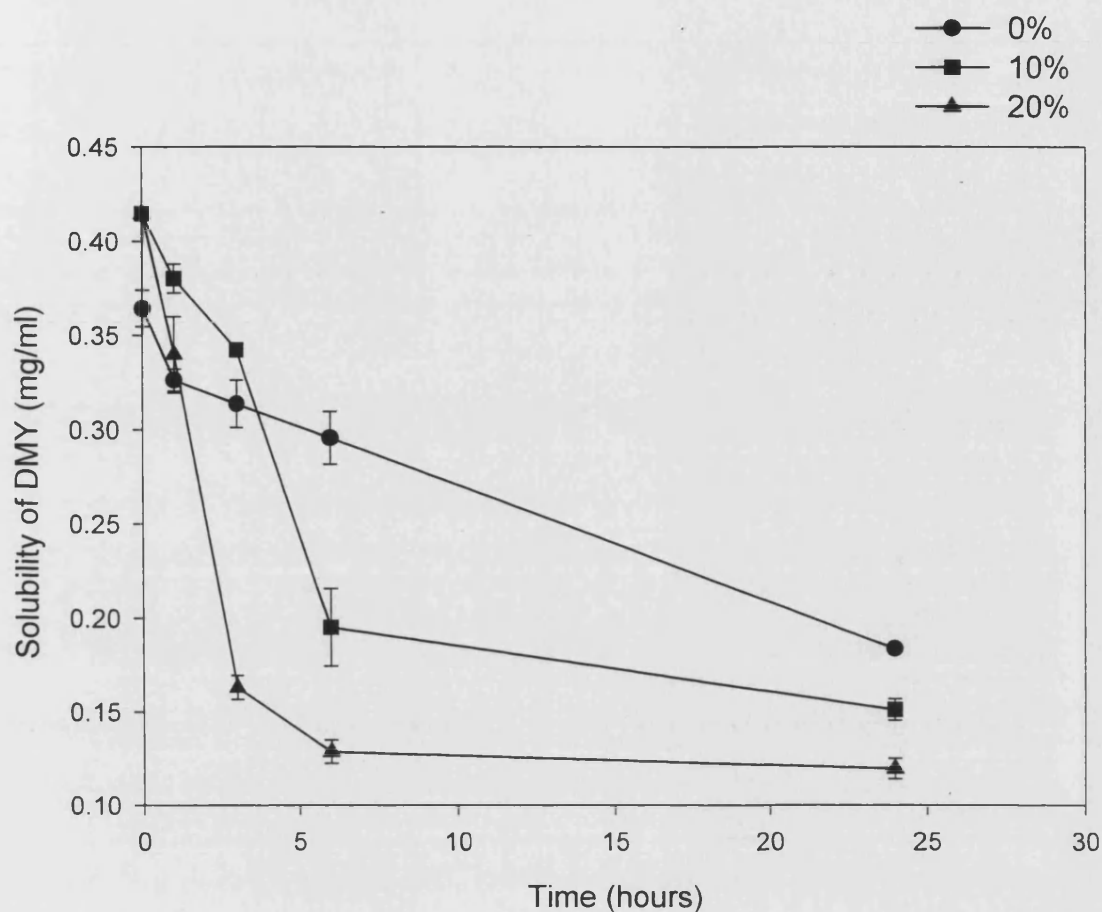
#### *Effect of Oil-Cosurfactant Ratio on the Solvency of Dispersions*

As was demonstrated in section 3.2, oil-cosurfactant ratio in the pre-concentrate mixture is an important parameter which determines the droplet size of the resultant emulsion. Nevertheless, in the case of hydrophilic surfactants with high solubilization capacity such as Cremophor RH 40 or EL, oil-cosurfactant ratio becomes also a

crucial factor in affecting the kinetics of drug crystallization after the dispersion of the oil-system. This becomes more evident in the case of high hydrophilic lipid formulations and in particular, when water-miscible cosolvent is included in the system. By increasing the oil fraction in oil-cosurfactant blend, we increase the non-polar hydrocarbon regions which interfere with water's hydrogen bonding network. As a result, water's ability to "squeeze out" non-polar compounds is reduced and hence the rate of crystallization is retarded.

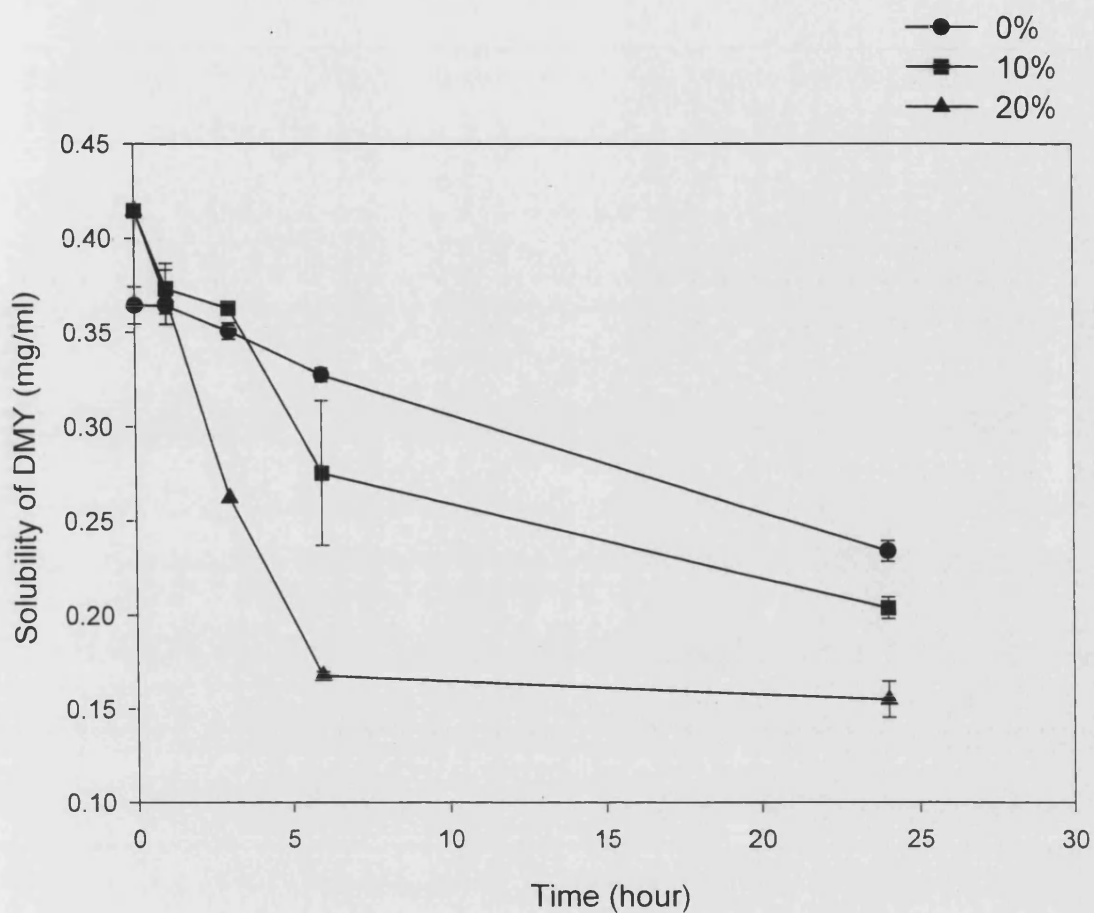
Figures 3.3.8 to 3.3.11 display the effect of oil-cosurfactant ratio in Miglyol 812/Imwitor 988-Cremophor RH40 system containing increasing concentration of PEG 400 on the solubilization behaviour of DMY after aqueous dispersion. For Miglyol 812-Imwitor 988 at ratios of 1:9 and 3:7 without PEG 400, gradual loss of DMY solubility was observed (Figures 3.3.8 and 9, respectively). Yet, in the case of 5:5 system (without PEG 400), DMY was maintained in supersaturated state for up to 24h from the initial event of emulsification (Figure 3.3.10). However, the inclusion of PEG 400 at only 10-20% (w/w) in these systems accelerated DMY precipitation. There had been progressive drop in the solubility of DMY due to drug crystallization as the concentration of PEG 400 in the formulation was increased. The dissolved amount of DMY within the pre-concentrate mixture is enhanced as more cosolvent is used in the system. On dispersion of the formulation and due to loss of solvent capacity as hydrophilic components diffuse into the aqueous phase, crystallization of DMY occurs depending on the extent the cosolvent was contributing to its solubilization within the oil mixture, see table 3.3.3.

For Miglyol 812-Imwitor 988 (1:9) system at 10% or 20% PEG 400 in the case of (3:9), more than half of the drug precipitated within the first 6h (Table 3.3.3), which suggests that precipitation from SEDDS containing a cosolvent is likely to occur within the lumen of the gut. In contrast, when the Miglyol 812 content was further increased in the oil blend as in (5:5) system, only 10 and 30% of the DMY dose came out of solution in the case of inclusion either 10 or 20% PEG, respectively (Table 3.3.3). Therefore, by increasing the weight fraction of Miglyol 812 in the oil-cosurfactant blend, the solvency of the lipid system to maintain the drug in solution after the dispersion of the formulation is enhanced and consequently, precipitation is retarded, see figure 3.3.11.



**Figure 3.3.8**

Effect of inclusion increasing concentration of PEG 400 in the Miglyol 812/Imwitor 988-Cremophor RH40 {70(10/90)30} system on the solubilization behaviour of DMY after Aqueous dispersion. 1g of each formulation containing approximately 40mg DMY was allowed to emulsify in 100ml water at 37°C for 15 minutes. Concentration of DMY in solution as function of time was quantified analytically by UV method.



**Figure 3.3.9**

Effect of inclusion increasing concentration of PEG 400 in the Miglyol 812/Imwitor 988-Cremophor RH40 {70(30/70)30} system on the solubilization behaviour of DMY after dispersion in water.

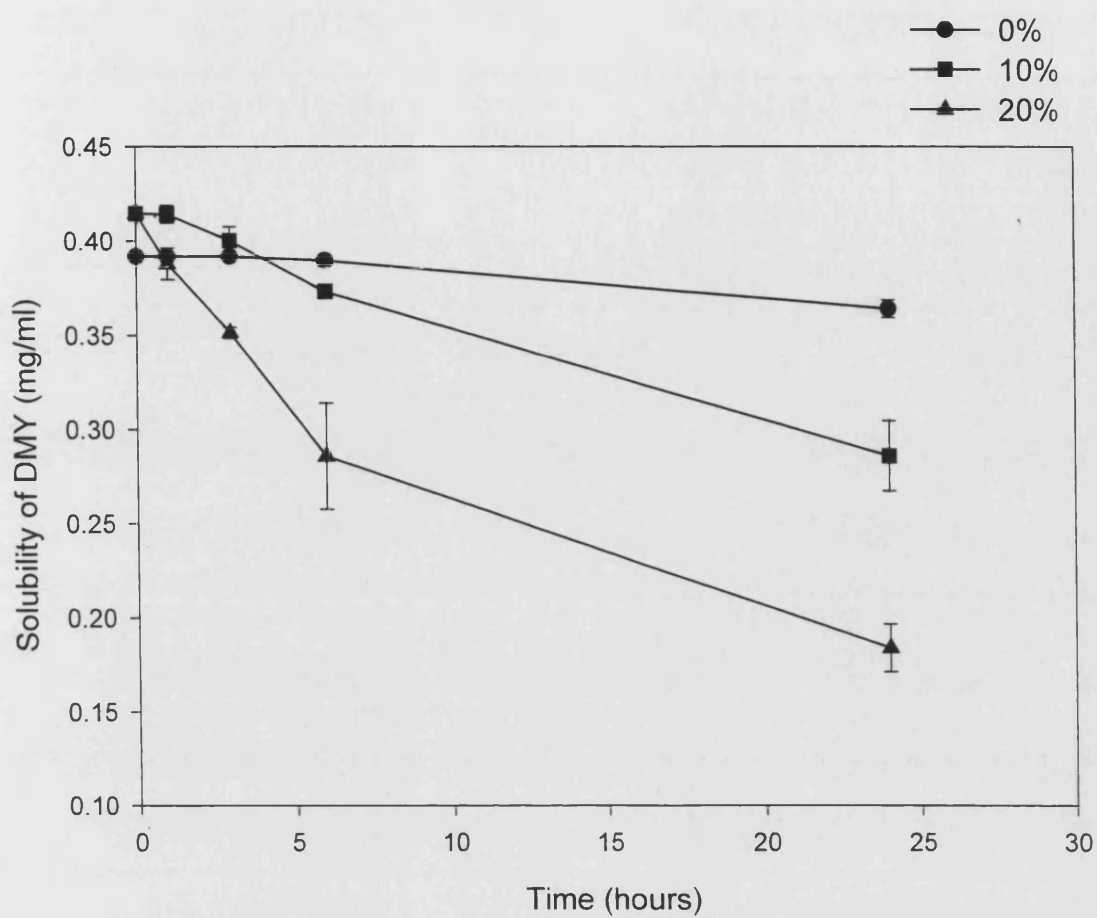


Figure 3.3.10

Effect of inclusion increasing concentration of PEG 400 in the Miglyol 812/Imwitor 988-Cremophor RH40 {70(50/50)30} system on the solubilization behaviour of DMY after aqueous dispersion.

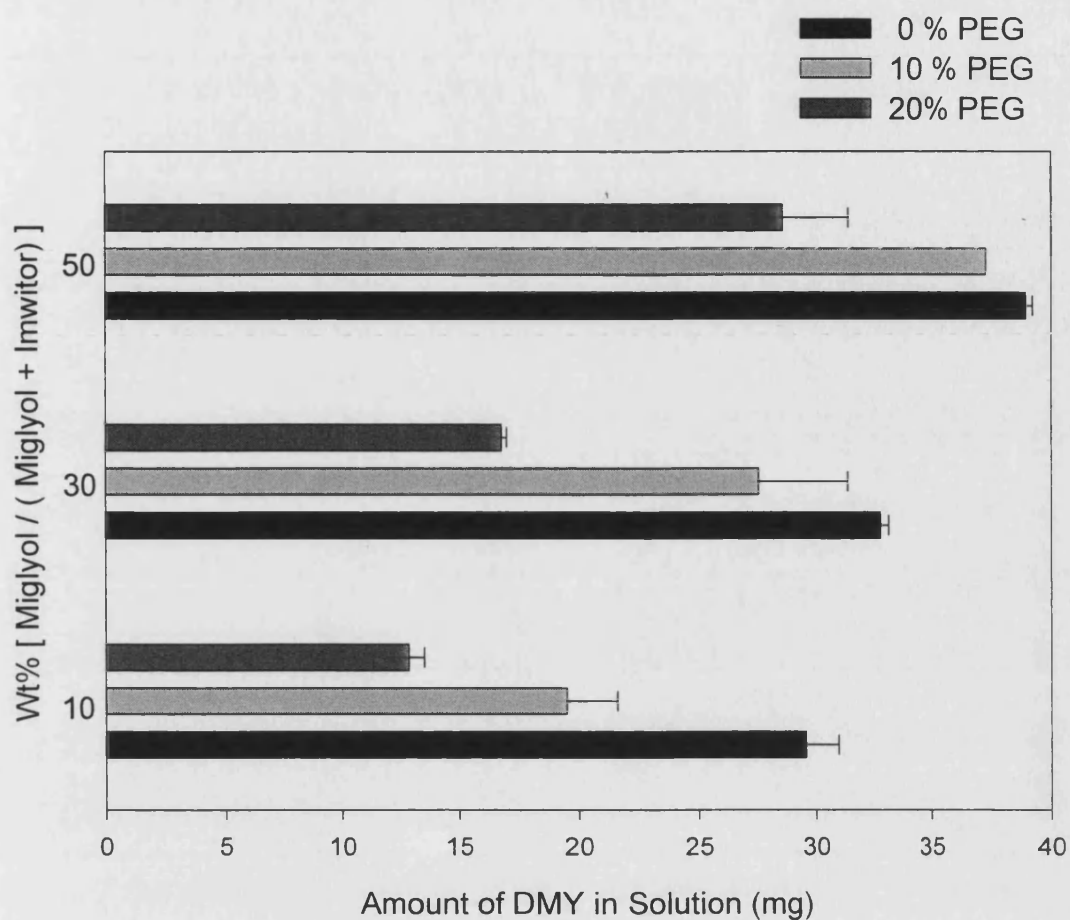


Figure 3.3.11

The effect of oil-cosurfactant ratio in the pre-microemulsion concentrate of Miglyol 812/Imwitor 988-Cremophor RH40 at increasing concentration of PEG 400 on the solubilization of DMY after 6h from the emulsification event. 1g of each formulation containing approximately 40mg DMY was allowed to emulsify in 100ml water at 37°C for 15 minutes.

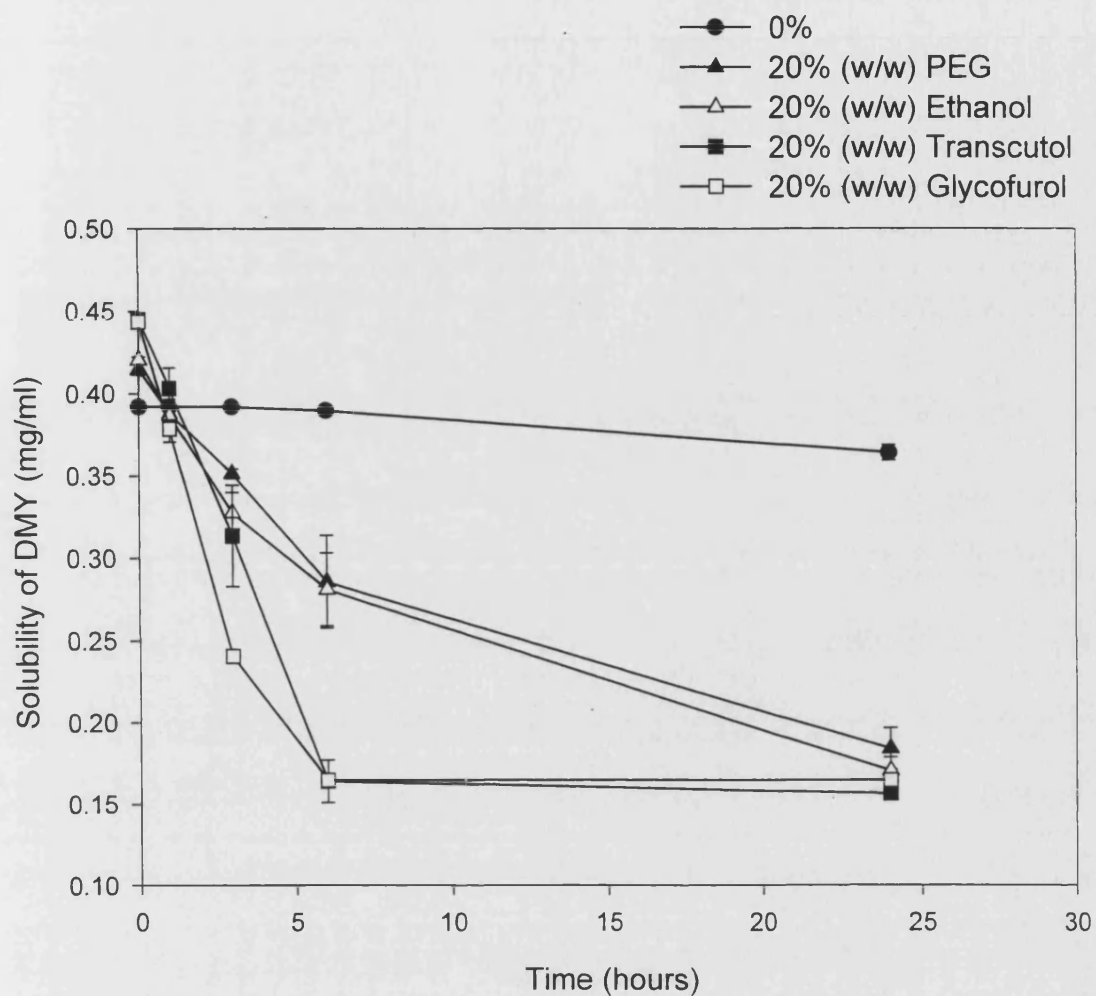


Figure 3.3.12 displays the effect of inclusion various cosolvents in the Miglyol 812/Imwitor 988 (5:5)-Cremophor RH40 system on the solubilization behaviour of DMY after the dispersion of the formulation. The solubilization profiles of DMY were comparable for oil systems containing either PEG 400 or Ethanol. On the other hand, in the case of using Transcutol or Glycofurol, almost equivalent profiles were also obtained yet with relatively high crystallization rate vis-à-vis formulations containing PEG 400 or Ethanol. This is attributed to the high solubilization power of these cosolvents as they are able to dissolve more DMY within the lipid formulation which tend to crystallize on dispersion.

**Table 3.3.3: The effect of oil-Cosurfactant ratio on the crystallization of DMY from the Miglyol 812/Imwitor 988-Cremophor system containing various percentages of PEG 400.** (Miglyol 812 is used as oil, Imwitor 988 as a cosurfactant and Cremophor RH 40 is the hydrophilic surfactant).

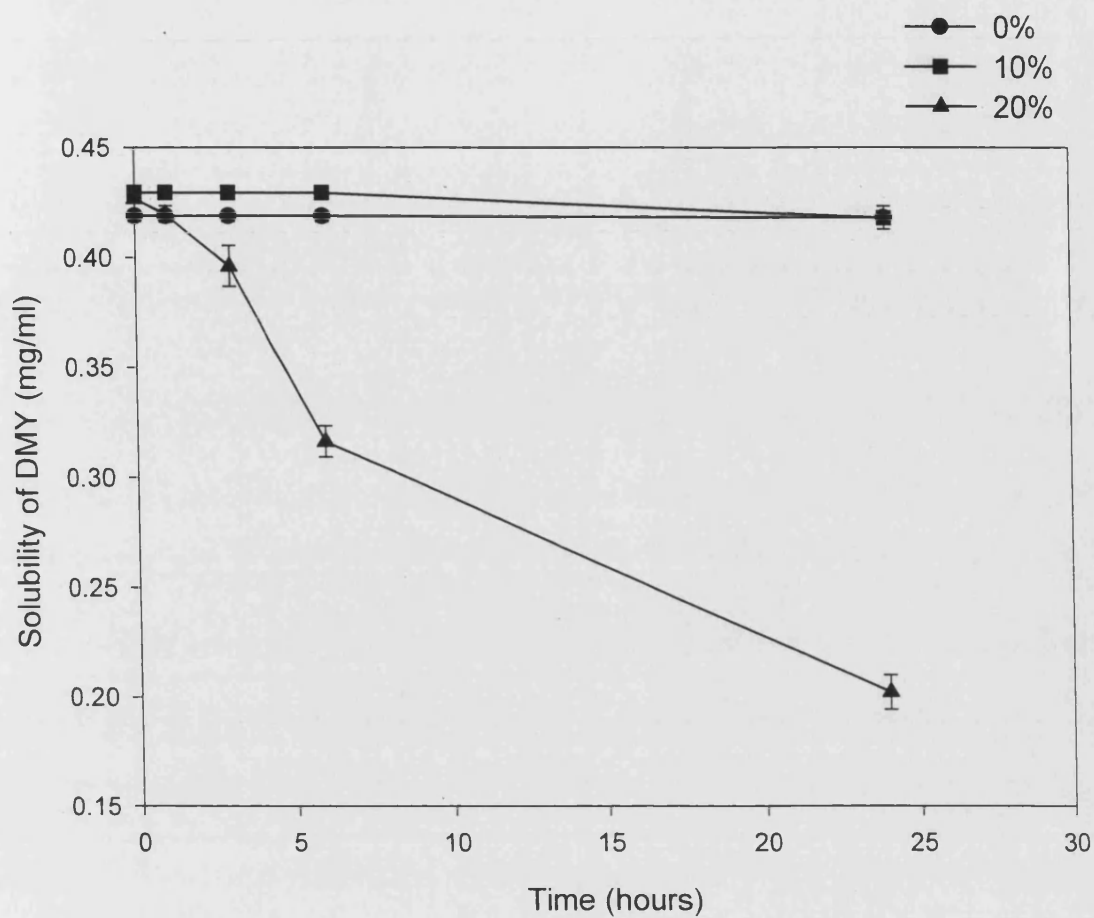
Oil : Cosurfactant	1:9	3:9	5:5
PEG 400 % (w/w)	Amount of precipitated DMY after 6h from dispersion (mg)		
0	6.88±0.54	3.72±0.61	≈ 0
10	21.95±2.4	13.94±3.44	4.32±0.17
20	28.58±0.91	24.54±0.53	12.88±3.14

Furthermore, the effect of including water-miscible cosolvent in Type II self-micro-emulsified lipid system, which is composed of water insoluble materials, on the crystallization of DMY after emulsification was also investigated and presented in figure 3.3.13. The solubilization profiles of DMY observed from Miglyol 812/Imwitor 988 (7:3)-Tagat TO system with and without any cosolvent have shown similar trend to the Miglyol 812/Imwitor 988 (5:5)-Cremophor RH40 system (Figures 3.3.13 and 3.3.10, respectively). The inclusion of 10% PEG in the system did not influence the solubilization behaviour of DMY yet, around 25% of the drug precipitated after 6h from dispersion when 20% PEG was included in the pre-concentrate (Figure 3.3.13). The migration of the cosolvent into the aqueous phase



**Figure 3.3.12**

Effect of inclusion water-soluble cosolvents with various solubilization powers ( $\sigma$ ) in the Miglyol 812/Imwitor 988 (5:5)-Cremophor RH40 system on the solubilization profiles of DMY.



**Figure 3.3.13**

Effect of inclusion increasing concentration of PEG 400 in Type II self-micro-emulsified lipid system (Miglyol 812/Imwitor 988-Tagat TO) on the solubilization behaviour of DMY after dispersion in water.

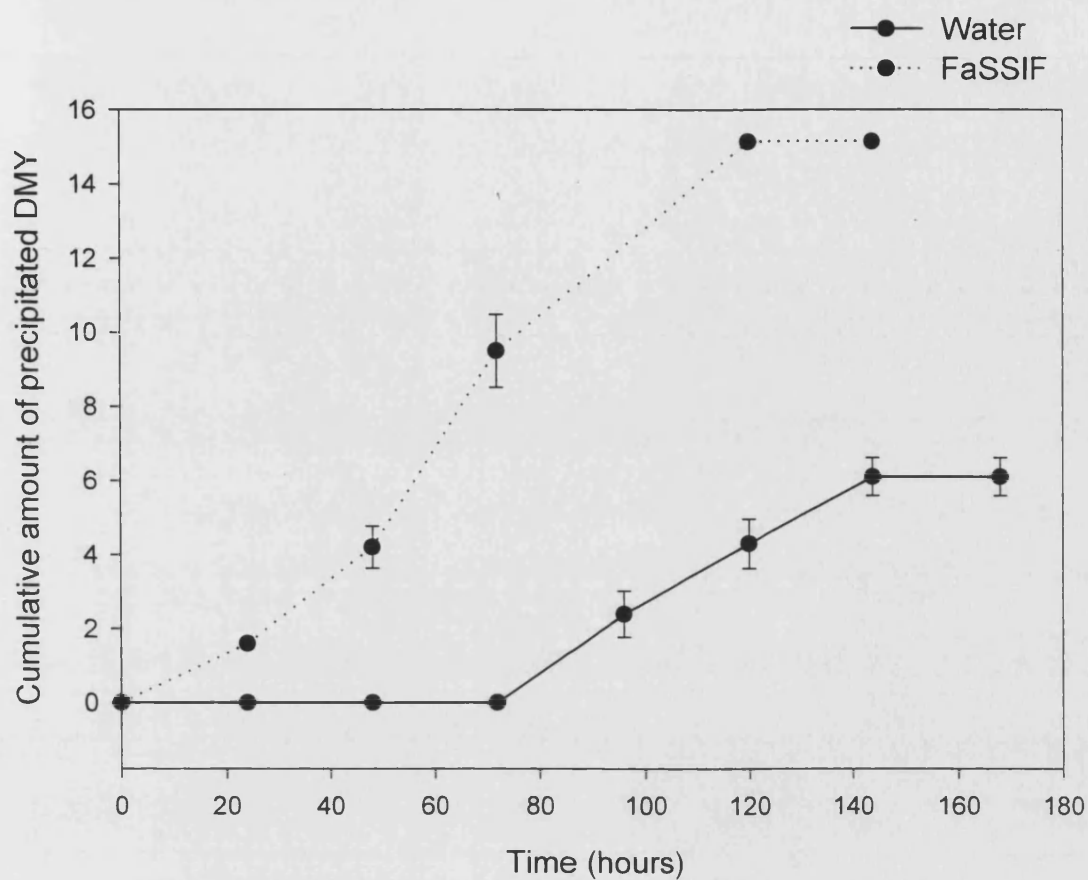
and large surface area of contact with water due to the formation of fine dispersion are the two main factors which affect the crystallization of DMY in this system.

#### *Effect of the ionic strength of Emulsification media*

In order to establish better in vitro-in vivo correlations for the oral administration of poorly water-soluble drugs, the composition, volume and hydrodynamics of the contents in the gastrointestinal lumen following the administration of the dosage form need to be accurately simulated <sup>[40]</sup>. Four media have been developed to simulate composition of the gastric and intestinal contents before and after ingested meal. Simulated Gastric Fluid (SGF) is used to simulate fasted gastric conditions, homogenized long-life milk (3.5% fat, pH 6.5) has been suggested to simulate fed state stomach, FaSSIF and FeSSIF to model fasted and fed state conditions in the small intestine, respectively.

In this study the effect of FaSSIF and FeSSIF media on the solubilization behaviour of DMY from Type II and Type III lipid class systems were investigated. In order to establish the importance of the bile salt-lecithin mixed micelles in the kinetics of drug crystallization from these systems, FaSSIF medium was prepared without bile salt and lecithin, see table 3 in chapter 2. This will give us an insight into the solubilization capacity of the endogenous surfactants and moreover will address the effect of electrolytes on the solvency of these lipid systems after dispersion.

Figure 3.3.14 depicts the crystallization of DMY from Miglyol 812/Imwitor 988 (7:3)-Tagat TO system after the emulsification in water or FaSSIF medium without bile salts-lecithin mixed micelles. As this figure shows, the emulsification of this system in FaSSIF accelerated the crystallization of DMY. At equilibrium which took up to 5 days to reach, around 15% of drug precipitated when the system was emulsified in water in comparison with 35% in the case of FaSSIF. The effect of electrolytes on the emulsification behaviour of this system without drug was thoroughly studied in section 3.1. Generally, electrolytes reduce the hydrophilicity of the non-ionic surfactants and hence they become more soluble in the oil phase. This will cause depression in the phase inversion temperature (PIT) of the system and eventually and phase separation.



**Figure 3.3.14**

Effect of ionic strength of the emulsification media on the crystallization of DMY as function of time from Type II self-micro-emulsified lipid system which contains water insoluble materials (Miglyol 812/Imwitor 988-Tagat TO 70(70:30)30). FaSSIF medium was used here without the bile salt-lecithin mixed micelles; see the text for further elaboration.

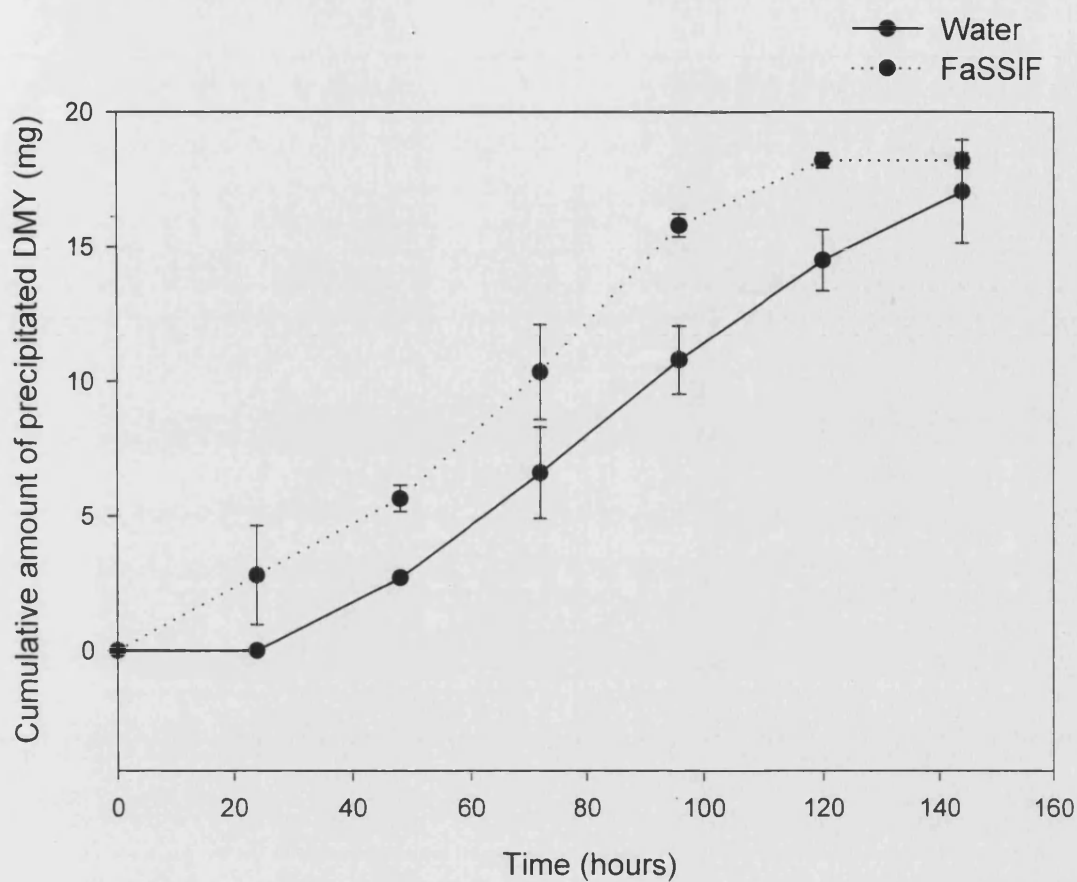
This effect will depend on the inclusion of high polar oil in the formulation like Imwitor 988 and moreover, the use of non-ionic surfactant with relatively low HLB such as Tagat TO. In this case, the reduction in the HLB of the surfactant incurred by the presence of electrolytes in the emulsification media will be sufficient to cause shift in its solubility from the aqueous to the oil phase which results in phase separation and eventually crystallization of the dissolved drug. However, for Miglyol 812/Imwitor 988 (7:3)-Tagat TO phase separation was not seen yet, there had been substantial increase in the droplet size due to the shift in Tagat To solubility. Nonetheless, by including more Imwitor 988 in the oil blend as in the case of Miglyol 812/Imwitor 988 (6:4)-Tagat TO total phase separation was observed after the dispersion in FaSSIF, which will be further elaborated in next section.

It is expected that the use of surfactant systems with high HLB value such as, Cremophor RH40 or EL will not be affected by the ionic strength of the emulsification media as the reduction in the surfactant HLB is not sufficient to induce phase separation. This was evident from the crystallization profiles of DMY from Miglyol 812/Imwitor 988 (5:5)-Cremophor system depicted in figure 3.3.15. Almost comparable trends were observed from the emulsification in water or FaSSIF. In both media more than 40% of DMY came out of solution at equilibrium, vis-à-vis 15% in the case of Miglyol 812/Imwitor 988 (7:3)-Tagat TO when emulsified in water (Figure 3.3.14). This is attributed to the fact that the former system contains a hydrophilic surfactant that tends to lose its solvent capacity after the dispersion.

As depicted in these figures (3.3.14 and 15) both formulations can take up to 5 days to reach equilibrium and that the drug can remain in a supersaturated state for up to 24h after the initial emulsification event. It could be argued that such products are unlikely to cause precipitation of the drug before the drug is absorbed and moreover, supersaturation might enhance absorption by increasing the thermodynamic activity of the drug <sup>[5]</sup>.

#### *The Effect of Bile Salt-Lecithin Mixed Micelles*

In the small intestine, drug solubility can be enhanced by the secretion of bile salts and other endogenous amphiphilic biliary components including lecithin and



**Figure 3.3.15**

Effect of ionic strength of the emulsification media on the crystallization of DMY as function of time from Type III self-micro-emulsified lipid system which contains a hydrophilic surfactant with relatively high HLB value (Miglyol 812/Imwitor 988-Cremophor RH40 70(50:50)30).

cholesterol. At concentrations higher than critical micelle concentration (CMC) these substances form mixed micelles which can enhance the solubilization capacity of the GI tract. Solubilization enhancement into simple bile salt micelles has been reported for many lipophilic compounds [53, 54, and 55]. Up to 100-fold increase in solubility has been observed upon addition of bile salts at physiological concentrations to aqueous media. Moreover, Dissolution rate for many lipophilic compounds was substantially improved in the presence of bile salts. Improve the wetting was the predominant mechanism for substances at log P values in the range of 1-2 [41]. However, in the case of highly lipophilic compounds such as danazol (log P 4.53), the increase in powder dissolution rate was attributed to the solubilization enhancement [56].

On the other hand, bile salt-lecithin mixed micelles was found to further enhance the solubility of lipophilic compounds [57, 58 and 59]. Addition of lecithin causes an increase in the molecular weight of micelles from 6000 to 150,000 Dalton [60] and hence more molar volume could be included into the palisade layer of the micelle. The extent of solubilization was shown to be influenced by the ratio of bile salts to lecithin [61]. Nonetheless, at high mixed micelles concentration enormous increase of the micellar diameter occurs. Therefore, a consequence of drug solubilization within micelles is a decrease in the apparent diffusion coefficient [62], since the effective diffusivity will be that of the micelle rather than of the drug monomer.

Mithani et al [58] studied the solubilization of a range of drugs by taurocholate solutions, and based on the drug log P and aqueous solubility developed good predictive estimates of the solubilization enhancement by bile salts. A linear correlation (Eq. 5) was observed between the logarithm of the solubilization ratio (SR), and log P.

$$[\text{SR}] = 0.64 \times \log [\text{P}] + 2.09 \quad (\text{Eq. 5})$$

Solubilization ratio (SR) can be defined as the ratio between the solubilizing capacity of bile salt micelles (SCbs) measured as moles drug /mole taurocholate and the solubilization capacity in water (SCaq). According to this model, the process of solubilization is entirely driven by the hydrophobicity of the drug. Yet, in addition to

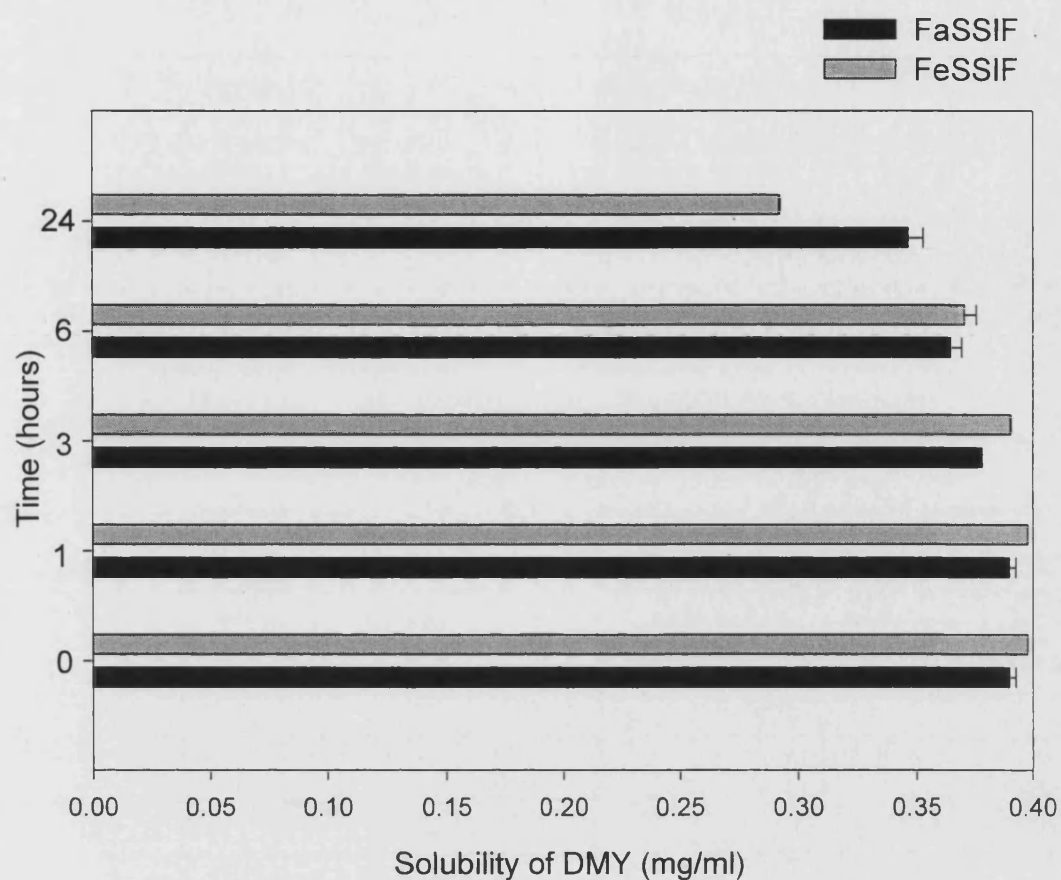


partitioning behaviour other factors such as, the drug molecular weight or shape, and the affinity for bile salt micelles can also play a role.

Data obtained by Solomon et al <sup>[59]</sup> for a range of steroids confirmed that shape is a factor which clearly can influence the extent of solubilization as the spatial orientation into the micelles available to the drug is limited. Long-chain esters of hydrocortisone such as hydrocortisone caprylate whilst being hydrophobic with log P 7.82 could not be incorporated into the micelles as efficiently as progesterone which is less hydrophobic (log P 4.22) and therefore, solubilization enhancement was found to be far more less in the former. Furthermore, the higher affinity of bile salt micelles for indomethacin as compared to phenylbutazone could not be explained on the basis of lipid solubility and molal volume. The lower interaction of phenylbutazone with bile salts was attributed to repulsion forces due to its carbon acid <sup>[63]</sup>.

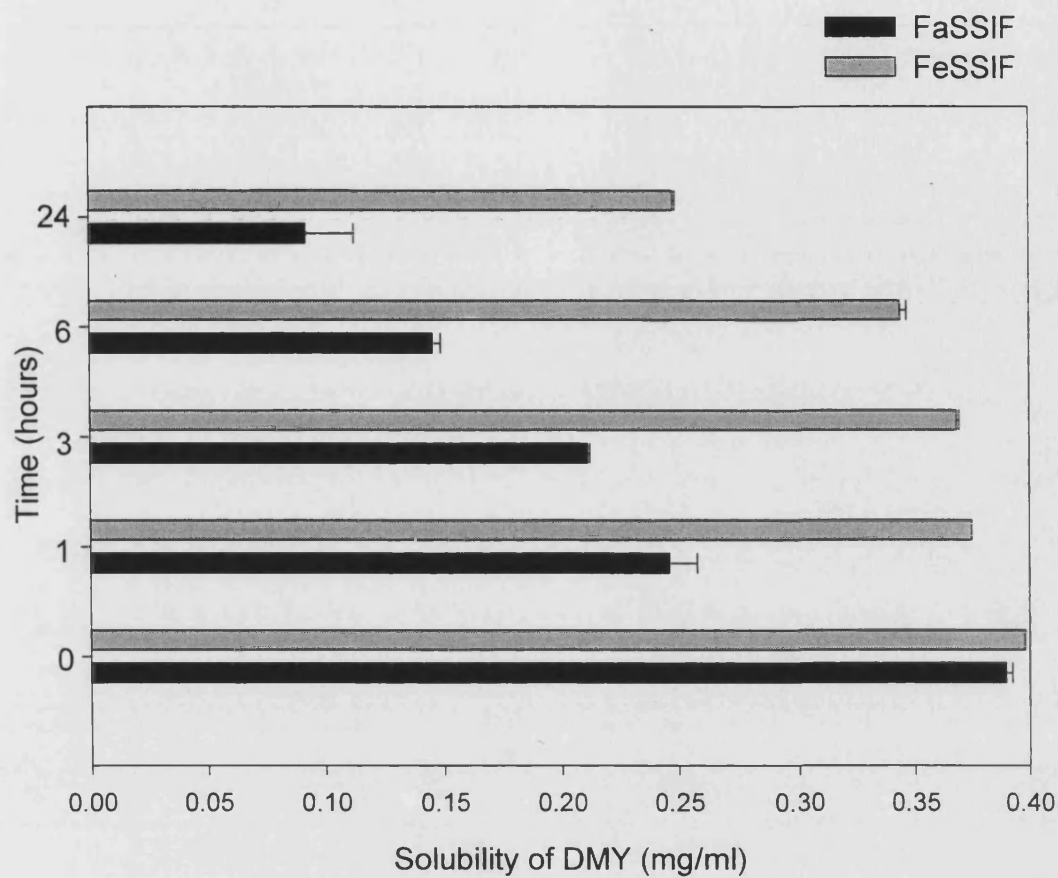
Figures 3.3.16, 17 and 18 depict the effect of various emulsification media on the solubilization behaviour of DMY from Type II and III lipid-class systems. For this study, FaSSIF medium was used without bile salt-lecithin mixture while FeSSIF contained mixed micelles of 15mM sodium taurocholate (NaTC)/3.75mM lecithin. For Miglyol 812/Imwitor 988 (7:3)-Tagat TO system, there was no significant difference in the solubilization profiles of DMY in FaSSIF vis-à-vis FeSSIF media for the first 6h from the emulsification event, see Figure 3.3.16. This is because loss of solvent capacity of the resultant dispersion in FaSSIF medium started to occur significantly 24h aftermath emulsification (Figure 3.3.14). Therefore, the effect of NaTC/lecithin mixed micelles in the solubilization enhancement of DMY could not be discerned.

Nevertheless, the solubilization behaviour of DMY from Miglyol 812/Imwitor 988 (6:4)-Tagat TO system was substantially influenced by emulsification in FaSSIF media, see figure 3.3.17. As was previously elaborated, gradual phase separation was observed after the dispersion of the former system in FaSSIF. Formation of an oil layer that is rich with hydrophobic surfactant and increasing amounts of dissolved DMY continued to develop on the top of the aqueous phase which contained few dispersed oil droplets. As a result, there had been drop in DMY solubility progressively with time after emulsification in FaSSIF (Figure 3.3.17). After 24h from the emulsification event and due to oil separation, almost 75% drop of the initial DMY solubility was observed. However, when the emulsification medium contained



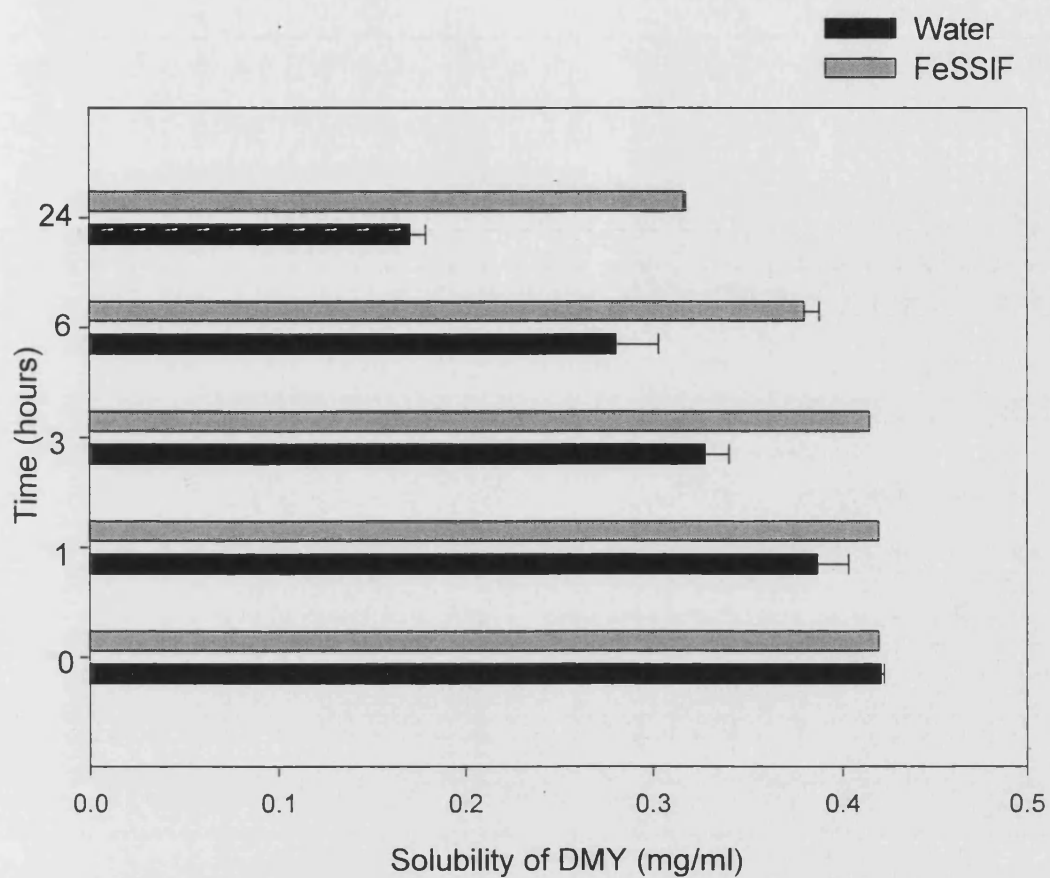
**Figure 3.3.16**

Effect of bile salt-lecithin mixed micelles on the solubilization capacity of Miglyol 812/Imwitor 988-Tagat TO {70(70/30)30} system after dispersion. 1g of each formulation containing approximately 40mg DMY was allowed to emulsify in 100ml FaSSIF (without bile salt mixed micelles) or FeSSIF medium which contained 15mM NaTC/3.75mM lecithin mixed micelles.



**Figure 3.3.17**

Effect of bile salt-lecithin mixed micelles on the solubilization capacity of Miglyol 812/Imwitor 988-Tagat TO {70(60/40)30} system after dispersion in various emulsification media.



**Figure 3.3.18**

Effect of bile salt-lecithin mixed micelles on the solvency of class IIIA lipid system containing a water-soluble cosolvent (Miglyol 812/Imwitor 988-Cremophor RH40-Ethanol 50(50/50)30/20) after dispersion in water or FeSSIF.

bile salt-lecithin mixed micelles to stimulate the intestine at fed state as in FeSSIF, the resulting dispersion was able to keep DMY in solution almost up to 24h after dispersion, see figure 3.3.17. This increase in DMY solubility is attributed to the enhancement of oil solubilization within the bile salt micellar system.

Similarly, as depicted in figure 3.3.18, in contrast to the loss of solvent capacity of Miglyol 812/Imwitor 988-Cremophor RH40 system containing 20% w/w Ethanol after dispersion in water, the emulsification in FeSSIF maintained the solubility of drug in solution. This indicates the capacity of NaTC/lecithin mixed micelles to prevent the drug from precipitation in the lumen of the gut during digestion of lipid formulation.

Despite the claim that the absorption of Cyclosporin A 'Sandimmune Neoral' is less affected by bile flow and pancreatin <sup>[64]</sup>, it is anticipated that the role of mixed micelles must become evident in the pharmacokinetics of Neoral whereby, at least a single oral dose of 200mg might be needed. Therefore, there is an immense need for practical methods to predict the fate of drugs after the dispersion of lipid systems in the GI tract.

It is worth noting here that loss of solvent capacity for all dispersions in FeSSIF was only observed 24h aftermath emulsification which might reflect the in-stability of mixed micelles with time. Yet, it is evident that the efficiency of dissolution and absorption for lipophilic compounds with  $\log P > 2$  are probably better in the fed state than fasted. In a study by Charman et al <sup>[65]</sup> in healthy human volunteers showed that both peak concentration and area under the curve were about three times higher when danazol was administered in fed than fasted conditions.

### 3.3.2. Concluding Remarks

The bioavailability enhancement of most oral lipid-based formulations depends on the ability of the oil vehicle to maintain the drug in solution after dispersion. Solvency of emulsions formed by self-emulsifying drug delivery system is an important parameter influencing the fate of dissolved drug after dispersion of the formulation. Physicochemical factors which determine the solvent capacity of these emulsions such as, hydrophilicity of lipid system, droplet size of the resultant dispersion,  $\log P$

of drug, ionic strength of the emulsification media and the bile salt-lecithin mixed micelles were sought for investigating the tendency of drug to precipitate after administration of an oily vehicle. In vitro methods, however, were used in this study in order to investigate the solubilization behaviour of the drug from dispersions formed by Type II and Type III lipid class systems in an attempt to predict the dynamic changes, which are expected to occur in the gut. Nonetheless, there is a clear need for developing methods for tracking the solubilization state of the drug in vivo.

Self-micro-emulsified lipid systems formulated with Miglyol 812, Imwitor 988 and Cremophor RH40 or Tagat TO lost solvent capacity on dispersion and were not able to keep the drug in solution at equilibrium. For several hours after dispersion crystalline drug could not be detected, and precipitation continued for several days until equilibrium was reached. In this case the drug is likely to be maintained in a supersaturated state for the period required for the absorption from the GI tract. Nonetheless, for pre-microemulsion systems containing Tagat TO, dispersions were found to hold more drug in solution at equilibrium than in the case of Cremophor RH40. Moreover, the Miglyol 812/Imwitor ratio in the pre-concentrate mixture appeared to influence the kinetics of drug crystallization. The inclusion of as little as 10-20% PEG in the lipid mixture accelerated drug precipitation. Up to half of the drug came out of solution within the first 6h, which suggests that precipitation from a SEDDS containing a cosolvent is likely to occur within the gut lumen. Yet, bile salt-lecithin mixed micelles appears to enhance to some extent the solubilization capacity of these systems after dispersion, though it is not clear whether the capacity of the mixed micelles would be sufficient to prevent drug precipitation in the gut during digestion of the lipid formulation.

Therefore, in order to maximize the solubilization capacity of lipid systems after dispersion and hence circumvent drug crystallization, pre-formulation studies are required to assess the viability of any lipid system. This includes; determination of drug solubility in the lipid matrix, optimization of factors which can influence the solubilization behaviour of the drug after dispersion such as oil-cosurfactant ratio, type of surfactant and the inclusion of water-miscible cosolvents, and more importantly the use of suitable emulsification media to stimulate the physiological conditions in the GI tract.

## Chapter 4

### Preparation of Solid Self-Micro-emulsified Lipid Systems (SMELS) for the Delivery of Hydrophobic Drugs

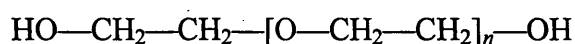
#### 4.1. Introduction

Sekiguchi et al. <sup>[1, 2]</sup> in the early 1960s reported that formulation of eutectic mixtures could lead to an improvement in the release rate and hence the bioavailability of poorly soluble drugs. Solid eutectic mixtures are usually prepared by rapid cooling of a comelt of two compounds to obtain a physical mixture of very fine crystals of the two compounds. Eutectic combinations such as sulphathiazole/urea <sup>[1]</sup> and chloramphenicol/ urea <sup>[2]</sup> exhibited examples for the preparation of poorly soluble drug in a highly water-soluble carrier.

The preparation of solid solution was then developed by Levy <sup>[3]</sup> and Kanig <sup>[4]</sup>. In contrast to a eutectic mixture, the dispersed component in a solid solution is molecularly dispersed. The term 'solid dispersion' refers to the dispersion of one or more active ingredients in an inert carrier or matrix at a solid state <sup>[5]</sup>. They are commonly used in the field of pharmaceutical technology in order to solve problems related to poor water solubility and poor bioavailability of active molecules, instability and dosing problems <sup>[6, 7]</sup>. The increase in aqueous solubility and dissolution of drugs can be attributed to the following factors <sup>[8, 9]</sup>: increase of the surface area available for dissolution by reducing the drug particle size, improvement of the apparent solubility of the solid compound under physiologically relevant conditions, and enhancement of the wettability and dispersability of the drug by the carrier which ensures sink conditions for dissolution. Moreover, the possible formation of a metastable dispersion that has a greater solubility resulting in faster dissolution rate.

The selection of the carrier and the method of preparation influence the properties of the resulting solid dispersion <sup>[5]</sup>. Among the popular carriers used in the formulation of solid dispersions are polyethylene glycols (PEG) <sup>[5, 10, 11, 12]</sup>, polyvinylpyrrolidone

(PVP) <sup>[13, 14, 15]</sup>, Cellulose derivatives (such as, hydroxypropylmethylcellulose (HPMC)) <sup>[16]</sup>, sugars <sup>[17, 18]</sup>, polyacrylates <sup>[19]</sup> and urea <sup>[2]</sup>. However, PEG based polymers have been extensively used due to their favourable solution properties, low toxicity and low cost. The general formula of these compounds is shown below:



where  $n$  represents the average number of oxyethylene groups ( $\text{OCH}_2\text{CH}_2$ ), i.e. the degree of polymerization.

Solid dispersion systems are usually manufactured by the hot melt method whereby, a physical mixture of the drug and the polymer is heated until the fluid state is reached and then the melt is cooled, or by the solvent method. Tachibani and Nakumara <sup>[20]</sup> were the first to dissolve both the drug and the carrier in a common solvent and then evaporate the solvent under vacuum to produce a solid solution.

In recent years there has been a growing interest in the lipid based formulations as a method to improve the bioavailability of poorly water-soluble hydrophobic drugs. The development of Cyclosporin Neoral<sup>TM</sup> demonstrated an excellent example of such successful approach. Self-emulsifying lipid systems are described as mixtures of oil and surfactant which emulsify in water under condition of gentle agitation, and can only be delivered in soft or hard gelatin capsules. Pouton <sup>[21]</sup> has recently classified these systems into type I, II and III with reference to the hydrophilicity of oil mixture, oil droplet size after aqueous dispersion and digestion by bile salts

In the present investigation solid self-micro-emulsified lipid systems (SMELS) were developed based on solid dispersion technology in an attempt to make a solid dosage form in order to replace costly and inconvenient soft gelatin capsule forms. Several solid formulations were developed using the hot melt method by the inclusion of either PEG 1000 or carnauba wax respectively in self-micro-emulsified lipid class systems representing type III or type II with and without a model drug. The physical stability and characteristics of these solid systems were investigated using differential scanning calorimetry and X-ray powder diffraction.



## 4.2. Characterization of Solid Dispersions

Various methods which have been used to characterize solid dispersions are listed in table 4.1. The most important methods among these are; thermoanalytical, X-ray diffraction, infrared spectroscopy and measurement of the release rate of the drug. These methods can be used to differentiate between solid solutions whereby drug is molecularly dispersed, solid dispersions in which drug is only partly molecularly dispersed and physical mixtures of drug and carrier. In the case of dispersions in which no crystallinity can be detected are considered to be molecularly dispersed. Therefore, the absence of crystallinity is used as a criterion to differentiate between solid solutions and solid dispersions <sup>[22]</sup>.

**Table 4.1: Methods for characterization of solid dispersions**

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Dissolution testing

Thermoanalytical methods: hot stage microscopy and differential analysis

Microscopic methods including polarization microscopy and scanning electron microscopy

Calorimetric analysis of the solution or melting enthalpy for calculation of entropy change

Spectroscopy methods, e.g. IR spectroscopy

X-Ray diffraction

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### 4.2.1. Differential Scanning Calorimetry (DSC)

Thermoanalytical methods include all that examine a characteristic of the system as a function of temperature. Of these, differential scanning calorimetry (DSC) and a related technique, differential thermal analysis (DTA), are the most highly effective thermal methods for studying the physical nature of a pure compound as well as its solid dispersion systems. These thermal techniques have been used in pharmaceutical industry for the detection and estimation of impurities <sup>[23, 24]</sup>, for the identification of polymorphic forms and solvates <sup>[25, 26]</sup>, for the assessment of molecular interactions

occurring between solid components of pharmaceuticals, <sup>[27, 28]</sup> and the rapid evaluation of the compatibility of drug substances with excipients <sup>[29, 30]</sup>.

The techniques of DTA and DSC are linked together viz. both are concerned with the measurement of energy changes in a substance. The word differential emphasises that measurements involve both a test sample and a reference material; in DTA it is proportional to the temperature difference ( $\Delta T = T_s - T_r$ ) while both are subjected to the same heating program, and in DSC to the differential thermal energy  $dq/dt$ .

DSC is usually regarded as quantitative technique in which endothermic or exothermic phase transformations (energy required and produced) are detected for all processes. The usual method of measurement is to heat the reference and test samples while keeping the temperature of the two identical throughout the controlled temperature program ( $\Delta T = T_s - T_r = 0$ ). If an energy-requiring phase transition occurs in the test sample (endothermic process), extra heat is applied to this sample so that its temperature increases at the same rate as in the reference. The additional heat required is recorded and used to quantitate the energy of the phase transition. In DSC, endothermic responses are usually represented as being positive ( $+\Delta H$ ), i.e. above the baseline, corresponding to an increased transfer of heat to the sample compared to the reference. Exothermic transitions ( $\Delta H$  negative) such as, oxidation or conversion of one polymorph to a more stable polymorph can also be detected however, the response will be in the opposite direction.

Lack of a melting peak in the DSC of a solid dispersion indicates that the drug exists in an amorphous rather than a crystalline state. The degree of crystallinity can be calculated for systems in which the drug is partly amorphous and partly crystalline. Nonetheless, crystallinities of less than 2% cannot generally be detected using DSC <sup>[31]</sup>.

#### 4.2.2. X-Ray Diffraction

A crystal consisting of atoms arranged in a pattern which is repeated regularly in a three dimensions, acts as a three dimensional diffraction grating for X-rays with wavelengths from about 0.5-2.5 Å <sup>[32]</sup>. The X-rays interact with the atoms (more

precisely, with their electrons) present in the crystal and the waves of scattered X-rays reinforce one another in certain orientations. Bragg has shown that reinforcement will occur when the rays diffracted from parallel planes are in phase with one another, i.e. when the path difference is an integral number of wavelengths. As depicted in figure 4.1 this can be represented by :

$$n\lambda = 2d \sin \theta \quad (4.1)$$

where  $\lambda$  is the wavelength,  $d$  is the distance between successive planes,  $\theta$  is the angle of incidence and reflection of the X-rays, and  $n$  is an integer.

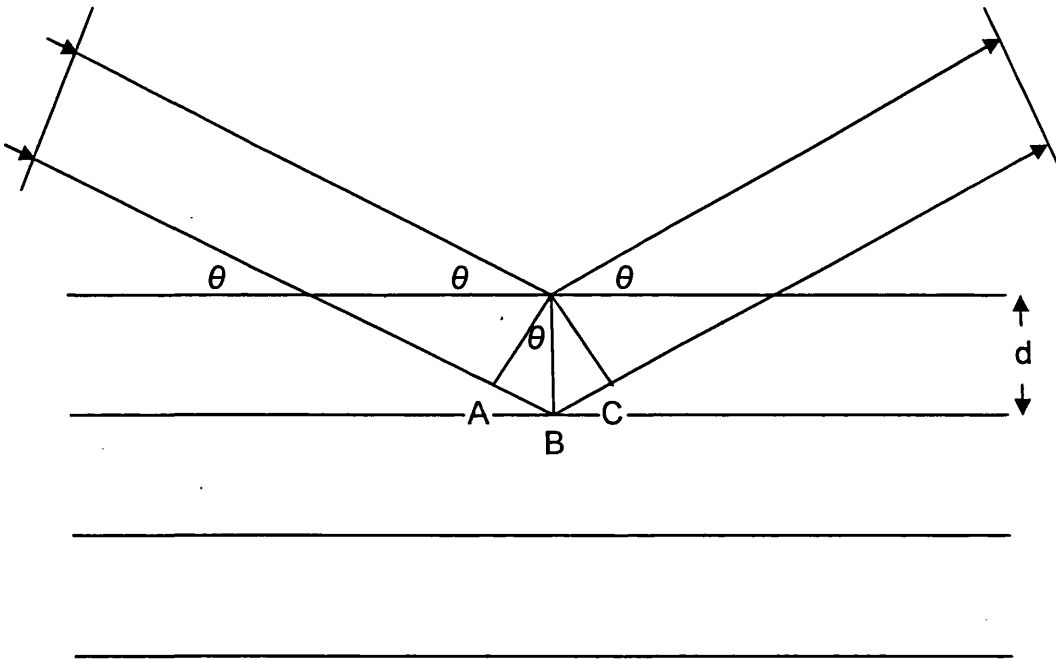


Figure 4.1

Bragg Law: Reinforcement of the diffracted rays from successive planes of a crystal occurs when the path difference is equal to an integral number of wavelengths ( $n\lambda$ ), i.e.  $AB + BC = n\lambda$ , where  $n$  is an integer. Since  $AB = BC = d \sin \theta$ ,  $n\lambda = 2d \sin \theta$ .

The principal behind X-ray diffraction is that when an X-ray beam is applied to the sample, interference bands can be detected. The angle at which the interference bands can be detected depends on the wavelength applied and the geometry of the sample

with respect to periodicities in the structure. The X-ray diffraction pattern of the single crystal or powder type can be recorded and interpreted using powder cameras or most efficiently by a counter diffractometer. In the powder camera the intensity of the diffracted X-radiation is measured by the degree of blackening the radiation produced on a photographic film. On the other hand, the intensity in the case of a counter diffractometer is measured directly in terms of the rate at which the diffracted photons trigger the counting device.

Crystallinity in the sample is reflected by a characteristic fingerprint region in the diffraction pattern. Due to the specificity of the fingerprint, crystallinity in the drug can be separately identified from crystallinity in the carrier. Hence, it is possible with X-ray diffraction to differentiate between solid solutions, in which the drug is amorphous, and solid dispersions, in which it is at least partly present in the crystalline form, irrespective whether the carrier is amorphous or crystalline. However, crystallinities of under 5-10% cannot generally be detected with X-ray diffraction <sup>[22]</sup>.

### **4.3. Materials and Methods**

#### **4.3.1. Materials**

Miglyol 812<sup>®</sup> (Medium chain triglycerides) and Imwitor 988<sup>®</sup> (Glycerol mono-/di-caprylate) were obtained from Condea Chemie GmbH, Hüls. Tagat<sup>®</sup> TO (polyoxyethylene-(25)-glyceryl trioleate, HLB 11.3) (Goldschmidt Chemical, Germany) and Cremophor RH 40 (polyoxyethylene-(40)-hydrogenated castor oil, HLB 14-16) (BASF Corporation) were used as nonionic surfactants to prepare with Miglyol 812 and Imwitor 988 formulations representing type II and III lipid class systems, respectively. Polyethylene glycol (PEG 10000) purchased from Sigma and Carnuba wax from Aldrich, were used as carriers for solid dispersions. n-Butyl p-hydroxy benzoic acid (BPHBA) also known as butyl parabens was used as a model lipophilic weak acid (log P= 3.24 and pKa 9.03), and was obtained from Sigma. Ethanol 96% w/w GPR, was purchased from BDH Chemicals and water was obtained from a Milli-Q (Millipore, Milford, MA) water purification system.

### 4.3.2. Methods

#### 4.3.2.1. Formulation of Self-micro-emulsified systems

Self-emulsifying systems representing type II and III lipid class formulations were prepared by mixing blends of Miglyol 812 and Imwitor 988 at ratios of 1:9, 3:7, 5:5, or 7:3 (w/w) with 30% (w/w) of either Tagat TO or Cremophor RH40, respectively. Stock mixtures of oil and surfactants to make formulations of 50g were accurately weighed into screw-capped glass bottles with tight closures followed by vortexing. Because of the waxy nature of Cremophor RH40, it was first melted in a water bath at 40°C before it was added to the oil mixture. The glass bottles were held at 50 °C in a thermostated water bath for 2 minutes before lipid mixtures were thoroughly vortexed. Lipid formulations were then left to equilibrate over night in an oven set up at 25°C.

#### 4.3.2.2. Phase behaviour of Self-emulsified Oil mixture-Carrier System

Oil mixtures containing various blends of Miglyol 812/Imwitor 988 at 30% w/w of Cremophor RH40 (type III systems) were added to increasing concentration of PEG 10000 (5-50% w/w). Increasing concentrations of carnauba wax (solid carrier) were used in the case of oil formulations containing blends of Miglyol 812/Imwitor 988 at Tagat TO concentration of 30% w/w (type II systems). The oil-carrier mixtures were then heated at 80°C in a water bath. When the carrier was completely molten and the obtained mixture was found to be homogenous, the molten mixture was left to cool down at 4°C in a refrigerator for solidification. Phase behaviour study was conducted by visual observation of the appearance of final products. Three types of mixtures were formed; pasty (semi-solid); solid glossy formulations; and immiscible two-phase mixtures, they were denoted P, S and II, respectively.

#### 4.3.2.3. Preparation of Solid Self-Micro-emulsified Lipid Systems (SMELS)

Self-micro-emulsified lipid systems, which form on aqueous dilution dispersions of <50nm, composed of blends of Miglyol 812/Imwitor 988 at ratios of 1:9 or 3:7 and

Cremophor RH40 at concentration of 30% w/w were added to varying amounts of PEG 10000 32.5 or 22.5% w/w, respectively. For systems composed of Miglyol 812/Imwitor 988 (7:3) and Tagat TO at concentration of 30% w/w, carnauba wax was used and was included at percentage of 30% w/w. Mixtures were then heated in a water bath at 80°C. After the carrier was completely molten, the drug (BPABA) was added at concentrations of (0-20% w/w) at the same temperature to allow the fusion of drug into the mixture. When the molten mixture was found to be homogenous, it was placed in a refrigerator at 4°C for rapid solidification.

#### **4.3.2.4. Solubility of BPHBA in the Lipid Formulations**

The model drug (BPHBA) was added in excess to the lipid formulations. Lipid suspensions were then vortexed for 3 minutes and then stored in a controlled temperature oven at 25°C for 24 hours to reach saturation, samples were vortexed in between. Oil suspensions were then transferred into 2ml eppendorphs and centrifuged at maximum speed (13000g) for 10 minutes. The clear saturated oil solution was then removed and assayed analytically by UV spectrophotometry (Perkin-Elmer Lambda 7, UV/VIS Spectrophotometer) at  $\lambda_{\max}$  of 252nm.

#### **4.3.2.5. Differential Scanning Calorimetry (DSC)**

Differential scanning calorimetry (DSC) measurements for various solid dispersions (see table 4.2) were carried out using a DSC-2910 differential scanning calorimeter (TA Instruments, Inc. UK) equipped with a liquid nitrogen sub-ambient accessory. Samples (6-8mg) were accurately weighed into 50  $\mu$ l aluminum pans and hermetically sealed. The samples were heated from 20 to 80°C at rate of 10°C/min under a dry nitrogen gas purge.

#### **4.3.2.6. X-Ray Powder Diffraction (XRD) Analysis**

The physical state of BPABA in PEG 10000, Carnauba wax and various SMELS formulations (see table 4.2) were evaluated with X-ray powder diffraction. Diffraction patterns were obtained on a Philips PW 1710 powder X-ray diffractometer using with

a radius of 173mm, using nickel-filtered Cu-K $\alpha$  radiation, a voltage of 40kV and a current of 25mA. Cross-sections of the solid dispersions were taken and held on a quartz plate for exposure to Cu-K $\alpha$  radiation. The samples were analysed at room temperature over an angle ranged from 2 to 60° of 2 $\theta$  with sampling intervals of 0.01° of 2 $\theta$  and a scanning rate of 1°/min.

#### 4.3.2.7. Physical Stability Study

Dispersions of varying drug concentrations in PEG 10000, Carnauba wax or SMELS (see table 4.2) were prepared as mentioned above. These dispersions were stored at 25°C for two years for physical stability investigation to detect any presence of crystalline BPHBA. Visual observations and XRD analysis were conducted over a period of 2 years. On the other hand, SMELS formulations without any drug (blank carriers) were monitored for chemical and physical stability periodically for 4 weeks.

**Table 4.2: Various Solid systems used in this investigation with the method of characterization; quantities and proportions of BPHBA and excipients used**

Self-Micro-emulsified oil mixture	Type of carrier system	Wt % of carrier	Wt % of BPHBA (drug)	Method of analysis	Period of stability study
-	PEG 10000	100-80	0-20	DSC, XRD	2-years
1	PEG 10000	22.50	0-20	DSC, XRD	4-weeks
1	PEG 10000	32.5	0-20	DSC, XRD	4-weeks
2	PEG 10000	32.5	0	DSC	4-weeks & 2-years
3	PEG 10000	22.5	0-20	DSC, XRF	2-years
-	Carnauba	100-80	0-20	XRD	-
4	Carnauba	30%	0-20	XRD	2-years

Oil mixture 1 = Miglyol/Imwitor/Cremophor RH40 70(10/90)30

Oil mixture 2 = Miglyol/Imwitor/Cremophor RH40 70(20/80)30

Oil mixture 3 = Miglyol/Imwitor/Cremophor RH40 70(30/70)30

Oil mixture 4 = Miglyol/Imwitor/Tagat TO 70(70/30)30

## 4.4. Results and Discussion

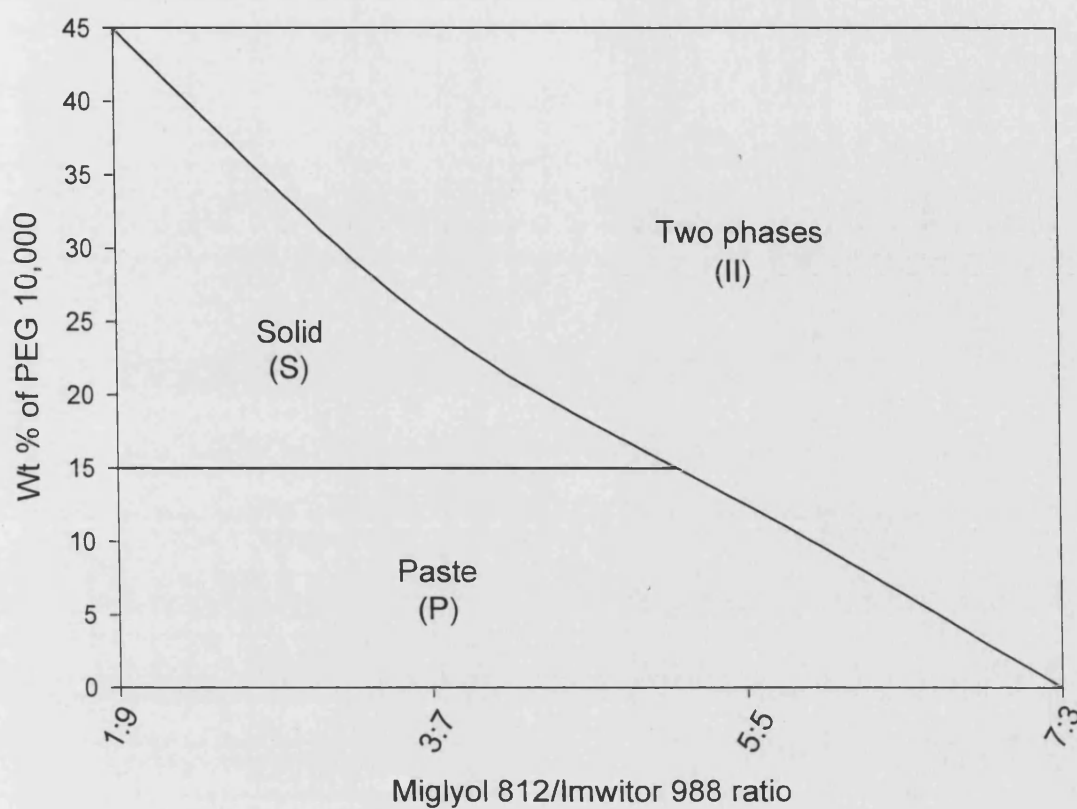
### 4.4.1. Phase Behaviour of Solid Self-micro-emulsified Lipid Systems (SMELS)

Various approaches have been attempted to improve the bioavailability of poorly water-soluble drugs. Such approaches are based on micronization of powders to increase the available area for dissolution, inclusion complexes such as cyclodextrines and derivatives, molecular dispersions in water-soluble polymers (PEG, PVP, HPMC, Poloxamer) and in non-electrolytes (urea, mannitol, sugars), micellar solubilization in surfactant systems (Tweens, Cremophors, Gellucires), liposomes, emulsions, microemulsions and self-emulsifying oil mixtures.

In this study, however, in order to develop a self-micro-emulsified system as a solid dosage form (tablet) in an attempt to circumvent costly and inconvenient soft gelatin capsule forms, the inclusion of water soluble polymer (PEG 10000) or carnauba wax in self-emulsified oil mixtures was investigated. PEG 10000 was included using the hot melt method in a hydrophilic type III lipid class system composed of various ratios of Miglyol 812/Imwitor 988-Cremophor RH40 to produce hydrophilic solid self-micro-emulsified lipid systems (*h*-SMELS). On the other hand, Carnauba wax due its lipophilic nature was incorporated in a hydrophobic type II oil class system composed of Miglyol 812/Imwitor 988-Tagat TO in order to obtain lipophilic solid carrier systems (*l*-SMELS).

Figures 4.2 and 4.3 depict the phase behaviour of inclusion PEG 10000 or Carnauba wax in oil mixtures representing type III and type II, respectively. Type III lipid class system which forms very fine dispersions on dilution with water consists of various oil blends of Miglyol 812/Imwitor 988 at a fixed concentration of Cremophor RH40 (30% w/w). Due to the fact that Imwitor 988 is polar oil as it is composed of equimolar of mono and di-glycerides, Miglyol 812/Imwitor 988 ratio in the oil system is the only factor which influences the hydrophilicity of the oil droplets. Therefore, as figure 4.2 shows the more Imwitor 988 in the oil mixture the more PEG 10000 could be incorporated in the system to form solid preparations (*h*-SMELS) before the system phase separates at relatively higher PEG concentrations.





**Figure 4.2:**

The effect of various oil blends (Miglyol 812/Imwitor 988) and increasing amounts of PEG 10000 on the phase behaviour of *h*-SMELS. Formulations of *h*-SMELS are composed of eutectic mixtures of the type III self-micro-emulsified oil mixture (Miglyol 812/Imwitor 988-Cremophor RH40) and the polymeric carrier PEG 10000. According to the nature of the final preparations, systems were classified into paste, solid and immiscible two-phase preparations, and were denoted P, S and II, respectively.

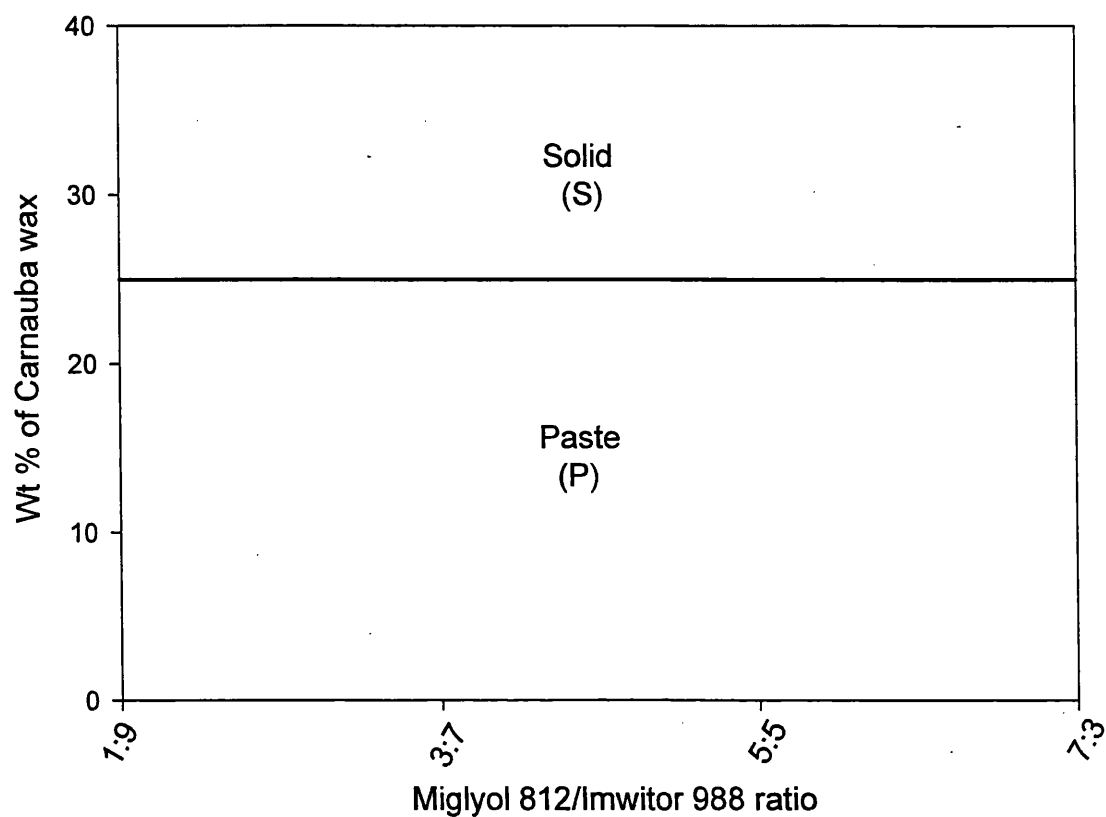
It is clear from figure 4.2 that the oil mixture in the system, depending on its degree of hydrophilicity, has a limited capacity for solubilizing certain concentrations of the water soluble polymer (PEG) above which the system tends to phase separate. Formulations containing <30% w/w of Imwitor 988 in the oil blend induced phase separation at all PEG concentrations.

For oil blends containing >50% Imwitor 988 a region of semi-solid systems was obtained at PEG concentrations of <15% w/w. As the percentage of PEG 10000 included in the system increases solidification occurs which suggests the formation of eutectic mixtures whereby, an intimate mixture of the self-emulsified oil is contained in the crystalline matrix of the polymeric carrier (PEG). As our interest in this investigation focuses on solid systems (SMELS) which might have the potential for tablet formulation, four preparations were selected for further characterization; {(Miglyol 812/Imwitor 988)-Cremophor RH40}<sub>oil mixture</sub> at ratios of 70(10/90)30 with PEG 10000 at concentrations of 22.5 or 32.5% w/w; oil mixture of 70(20/80)30 with PEG at concentration of 32.5% w/w; and 70(30/70)30 at PEG of 22.5% w/w.

It is worth noting here that similar phase behaviour was observed when PEG of higher molecular weight (20000) was used to formulate *h*-SMELS. However, a relatively slight shift in the phase boundary has occurred towards lower PEG concentrations in the case of PEG 20000 retrospective to PEG 10 000 as the former is considered to be more hydrophilic.

The phase behaviour of Miglyol 812/Imwitor 988/Tagat TO-Carnauba wax system is depicted in figure 4.3. This system has formed eutectic mixtures which are completely miscible in the liquid state at all Miglyol 812/Imwitor 988 ratios. This reflects the congruity of the degree of hydrophobicity of both the solid carrier and the self-emulsified oil mixture. As figure 4.3 shows semi-solid (paste) preparations are formed at carnauba wax concentration of <25% w/w. On the other hand, solid carrier systems can be obtained at carnauba wax concentration of >25% w/w.

The self-emulsified oil mixture {Miglyol 812/Imwitor 988-Tagat TO} is classified as type II lipid system according to the classification described by Pouton <sup>[21]</sup>, and is composed of water insoluble materials. Miglyol 812/Imwitor 988-Tagat TO at ratios



**Figure 4.3:**

The effect of various oil blends (Miglyol 812/Imwitor 988) and increasing amounts of Carnauba wax on the phase behaviour of Miglyol 812/Imwitor 988-Tagat TO-Carnauba wax carrier system. Formulations of *l*-SMELS are composed of eutectic mixtures of the type II self-emulsified oil mixture (Miglyol 812/Imwitor 988)-Tagat TO at ratios of 70(70/30)30 and carnauba wax (solid carrier) at percentage of 30% w/w.

of 70(70/30)/30 can be arbitrary called a self-micro-emulsified oil system as it produces on aqueous dilution very fine dispersions of oil droplet size  $>50\text{nm}$  (see chapter 3, section 3.1). Therefore, this system was selected to produce with carnauba wax lipophilic solid self-micro-emulsified lipid systems (*l*-SMELS).

Gelatin is added to w/o microemulsions to prepare microemulsion-based organogels (MBGs) which are rigid, optically transparent and thermoreversible. Organogels have been suggested as novel delivery vehicles for drugs and antigens <sup>[33]</sup>. MBGs have been prepared using various systems of surfactants and oils such as, Sodium bis(2-ethylhexyl)-sulfocuccinate (AOT)/Isooctane <sup>[34]</sup> and more recently Tween 80/IPM (isopropyl myristate) <sup>[35]</sup>. Formed microemulsions are incubated at  $50^{\circ}\text{C}$  and added to a solution of gelatin in water incubated at the same temperature; the resultant mixture is shaken and then allowed to cool to room temperature.

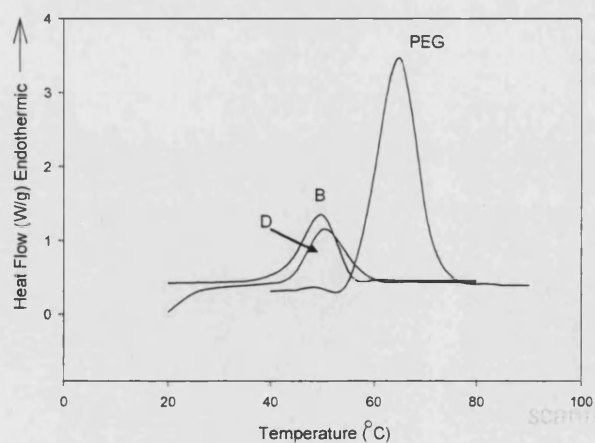
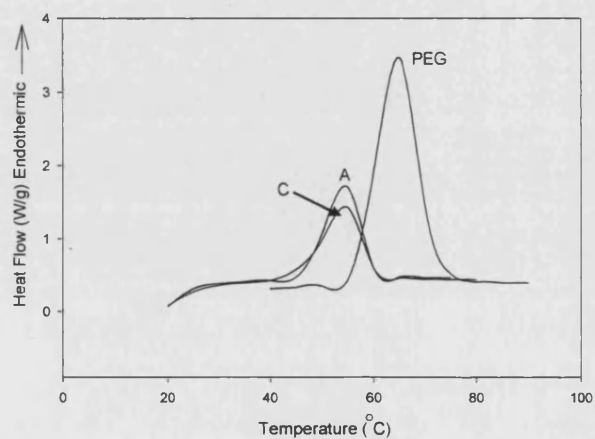
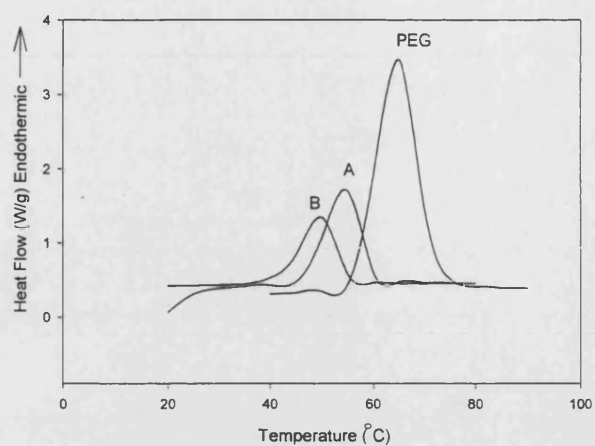
In this investigation, however, MBGs could not be prepared from the self-micro-emulsified systems of Miglyol 812/Imwitor 988-Cremophor RH40 or Miglyol 812/Imwitor 988 (7:3)/Tagat TO. Gelatin added to the parent microemulsions formed from these systems agglomerated and precipitated. This may be due to either chemical interaction or polarity difference between the o/w microemulsion and the gelatin solution.

#### 4.4.2. Physico-chemical Properties of SMELS

##### 4.4.2.1. *h*-SMELS

As mentioned earlier, solid carrier systems (SMELS) developed in this investigation were classified into hydrophilic (*h*) and lipophilic (*l*) depending on the solid carrier used and the type of self-micro-emulsified oil mixture. PEG 10000 with type III self-micro-emulsified oil system constitute *h*-SMELS while, Carnauba wax with type II self-micro-emulsified lipid system form *l*-SMELS.

The physical characterization of SMELS was carried out using differential scanning calorimetry (DSC) and X-ray diffraction (XRD). Figure 4.4 shows DSC thermograms for the pure polymeric carrier (PEG 10000) and four selected formulations of *h*-SMELS composed of different ratios of Miglyol 812/Imwitor 988 in the oil blend with varying concentrations of PEG 10000.



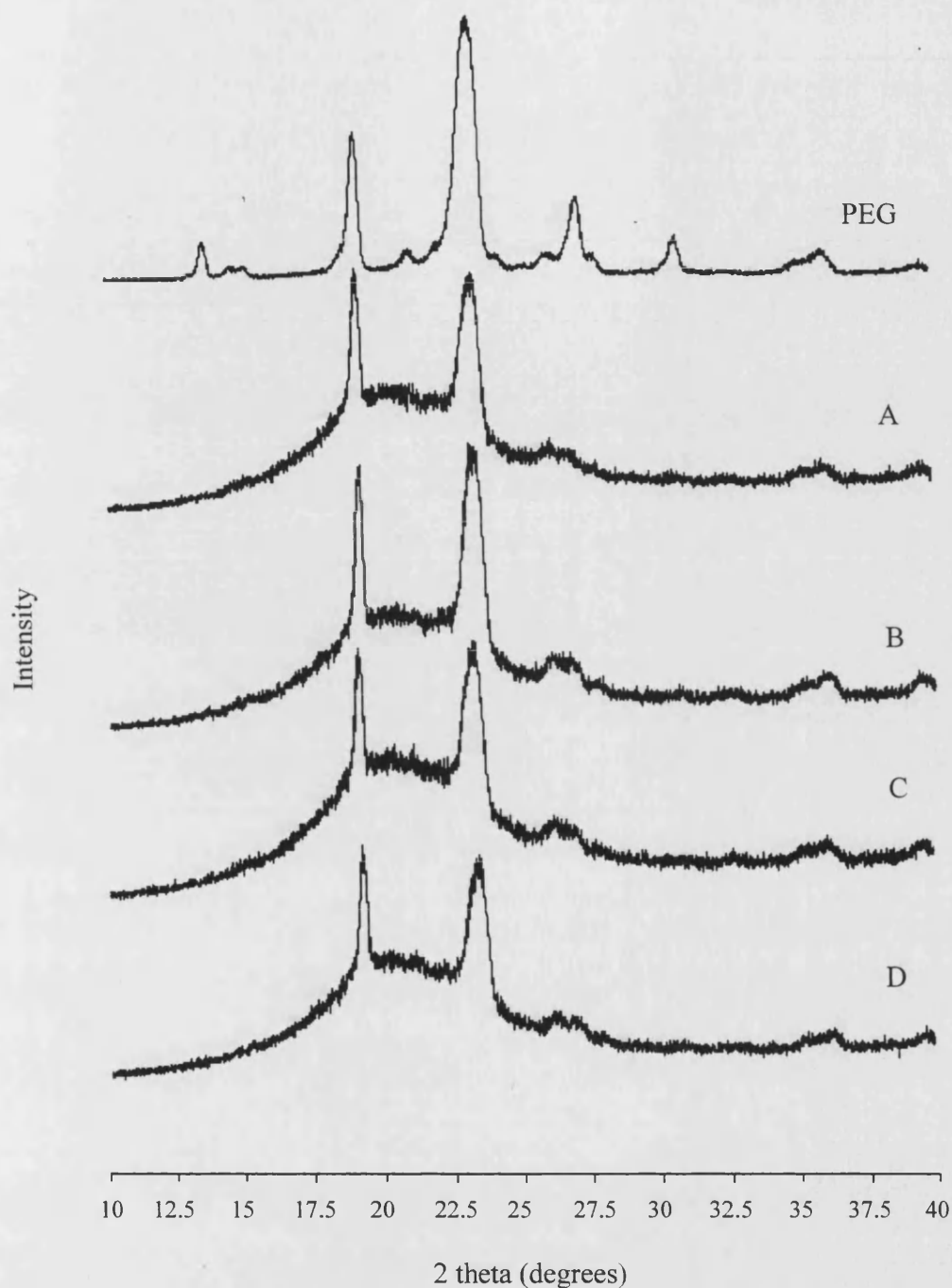
**Figure 4.4:** DSC thermograms of PEG 10000 and four selected formulations of *h*-SMELS; (A) ratios of oil 70(10/90)30 and PEG at 32.5% w/w, (B) oil ratios at 70(10/90)30 and PEG 22.5%, (C) oil ratios at 70(20/80)30 and PEG 32.5% and (D) oil ratios at 70(30/70)30 and PEG 22.5%. All oil mixtures are composed of Miglyol 812/Imwitor 988-Cremophor RH40.

Each thermogram of *h*-SMELS (A, B, C, and D, figure 4.4) has displayed a single melting endothermic peak similar to the blank carrier PEG yet, with shifts towards relatively lower melting temperatures and lower endothermic energies. The polymeric carrier PEG has a peak melting temperature of 63°C with an endothermic energy of 181 J/g whereas, formulations of *h*-SMELS have peak temperatures ranging from 49-54°C with enthalpies between 40 and 60 J/g, see table 4.3

There are two main elements in the composition of these solid carrier systems which can influence these thermal shifts; the percentage of included PEG and the ratio of Miglyol 812 (source of triglycerides) in the oil blend. At constant ratio of Miglyol 812/Imwitor 988, the more PEG used in these solid vehicles the higher the peak temperatures and endothermic energies these systems would have (compare peak A and B, see also table 4.3). This agrees fully with the phase behaviour depicted in figure 4.2 for *h*-SMELS whereby, the progressive increase of included PEG shifts the region of semi-solid preparations into region of solid systems due to corresponding increases in the melting point. On the other hand, at fixed concentration of PEG, a slight decrease in enthalpy is observed in systems containing relatively higher proportions of Miglyol 812 in the oil blend (compare peak A with C, and B with D).

This emphasizes the point we have mentioned earlier about the physical nature of these solid carrier systems whereby eutectic mixtures have formed. The constituents are completely miscible at elevated temperature and form one-phase liquid system, which on cooling, the polymeric carrier (PEG) crystallizes while solubilizing within its matrix the self-emulsified oil mixture.

In order to shed more light on these carrier systems (*h*-SMELS), XRD analysis was carried out. Figure 4.5 shows the X-ray diffraction patterns of pure PEG 10000 and four selected formulations of *h*-SMELS. Two characteristic peaks appeared in the X-ray diffractogram of PEG at a diffraction angle of  $2\theta$  19.16 and 23.25. Regardless their composition, all formulations of *h*-SMELS have produced diffractograms with characteristic peaks similar to that of the polymeric carrier (PEG) suggesting the formation of intimate mixtures whereby the self-emulsified oil is contained within the crystalline matrix of the PEG. The intensity of the peaks produced by *h*-SMELS corresponds to the percentage of PEG included in the system. Formulation B depicted in figure 4.5 contains more PEG and hence has produced peaks with relatively higher intensities comparable to the other preparations considered (A, C or D).



**Figure 4.5:**

X-ray powder diffractograms of pure polymeric carrier PEG 10000 and four selected formulations of *h*-SMELS; (A) ratio of oil 70(10/90)30 and PEG at 22.5% w/w, (B) oil ratios at 70(10/90)30 and PEG at 32.5% w/w, (C) oil ratios of 70(20/80)30 and PEG at 22.5% and (D) oil ratios at 70(30/70)30 at PEG concentration of 22.5%. All oil mixtures are composed of (Miglyol 812/Imwitor 988)-Cremophor RH40.

Thermal analysis using DSC on the stability of these blank carrier systems was carried out over a period of 4 weeks and the results are listed in table 4.3. Results suggest that these solid carrier systems are thermodynamically stable, rigid enough to stabilize the drug, are not susceptible to the aging processes and can maintain the drug molecularly dispersed in the amorphous state due their high content of oil. Therefore, these new carrier systems can form the bases of promising and genuine approach to formulate self-micro-emulsifying systems as a solid dosage form in order to deliver hydrophobic molecules. Furthermore, these solid carriers have produced in water fine dispersions of oil droplet size <50 nm which adds to the advantages of these systems in enhancing the bioavailability of poorly water-soluble drugs.

**Table 4.3: Thermal stability of four blank vehicles of *h*-SMELS over a period of 4 weeks. Systems are composed of the self-micro-emulsified oil mixture (Miglyol 812/Imwitor 988-Cremophor RH40) and varying amounts of PEG 10000.**

Formulation of <i>h</i> -SMELS	Onset temperature (°C)		Peak temperature (°C)		Enthalpy (J/g)	
	Week 1	Week 4	Week 1	Week 4	Week 1	Week 4
A	45.7	47.8	49.3	50.7	40.58	40.22
B	48.6	48	53.5	53.4	58.9	59.46
C	49.7	49.7	53.2	53	55.4	59
D	49.4	49.2	51.1	52.9	37.8	40.7

Formulation A = oil ratios of 70(10/90)30 at PEG 22.5% w/w

Formulation B = oil ratios of 70(10/90)30 at PEG 32.5% w/w

Formulation C = oil ratios of 70(20/80)30 at PEG 32.5% w/w

Formulation D = oil ratios of 70(30/70)30 at PEG 22.5% w/w

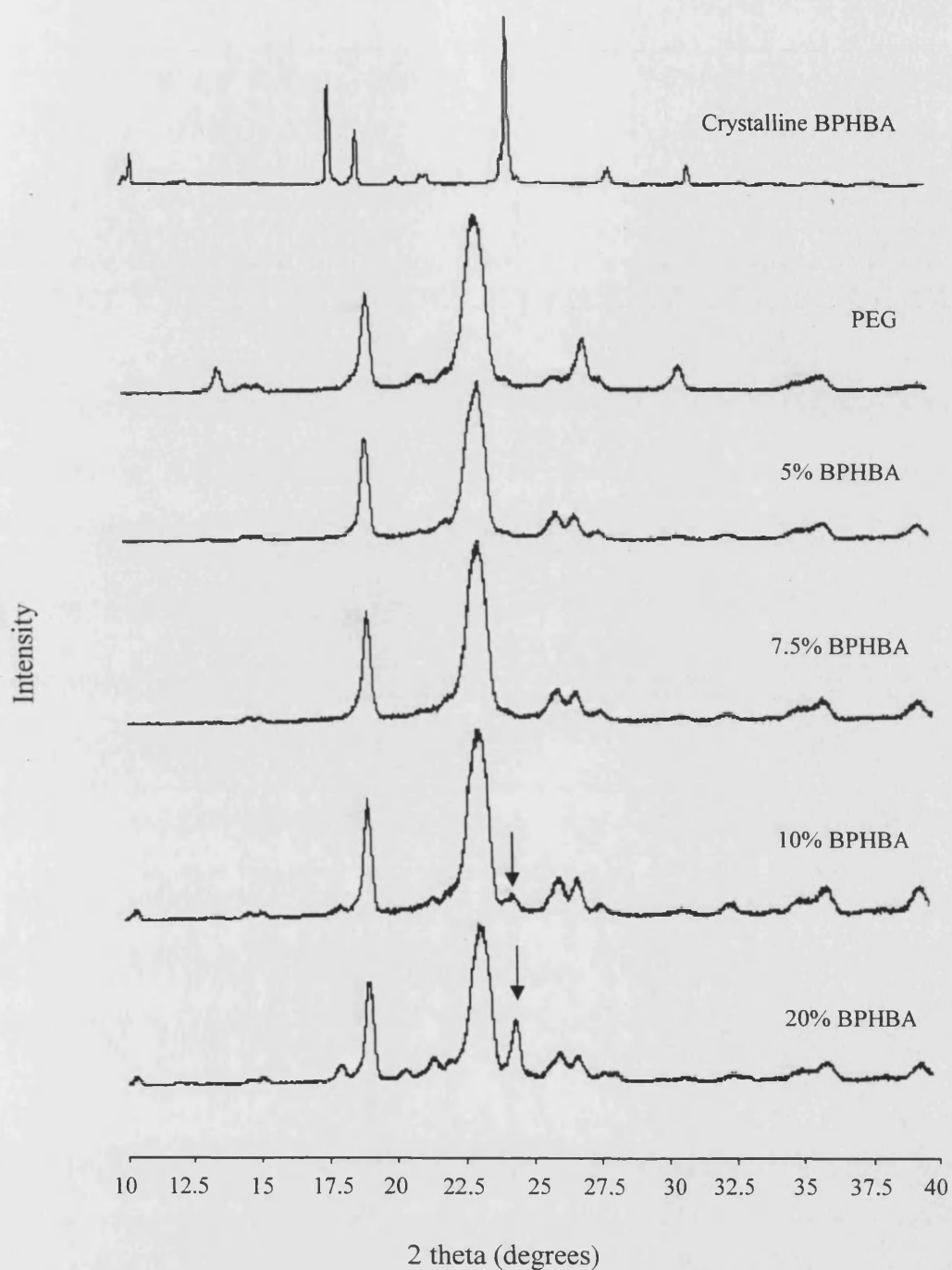
A potential advantage of solid dispersion systems is their capacity to present drugs to the gastrointestinal tract (GIT) in their high energy (metastable) amorphous state <sup>[34]</sup>, which is considered to be more highly soluble than that of most stable form (crystalline). The energy required to break up the crystalline structure of the drug before it can dissolve is therefore, not a limitation to the release of the drug from these systems. However, solid dispersions generally have limited loading capacity of active materials in the amorphous state, not more than 10% in best cases, after which contaminating crystalline material can be detected. Another limitation of the solid



dispersion systems is the tendency of the thermodynamically metastable amorphous drug to revert to the more stable crystalline form on aging. In a study on a griseofulvin-PEG 6000 solid dispersion system Chiou <sup>[35]</sup> reported that the amorphous material crystallized out on aging except when the drug concentration in the dispersion was  $\leq 5\%$ . Furthermore, Khoo et al. <sup>[34]</sup> showed that low percentages of Halofantrine (Hf) ( $< 10\%$ ) in Hf-PEG 6000 dispersions were more stable than those containing a higher percentage of Hf

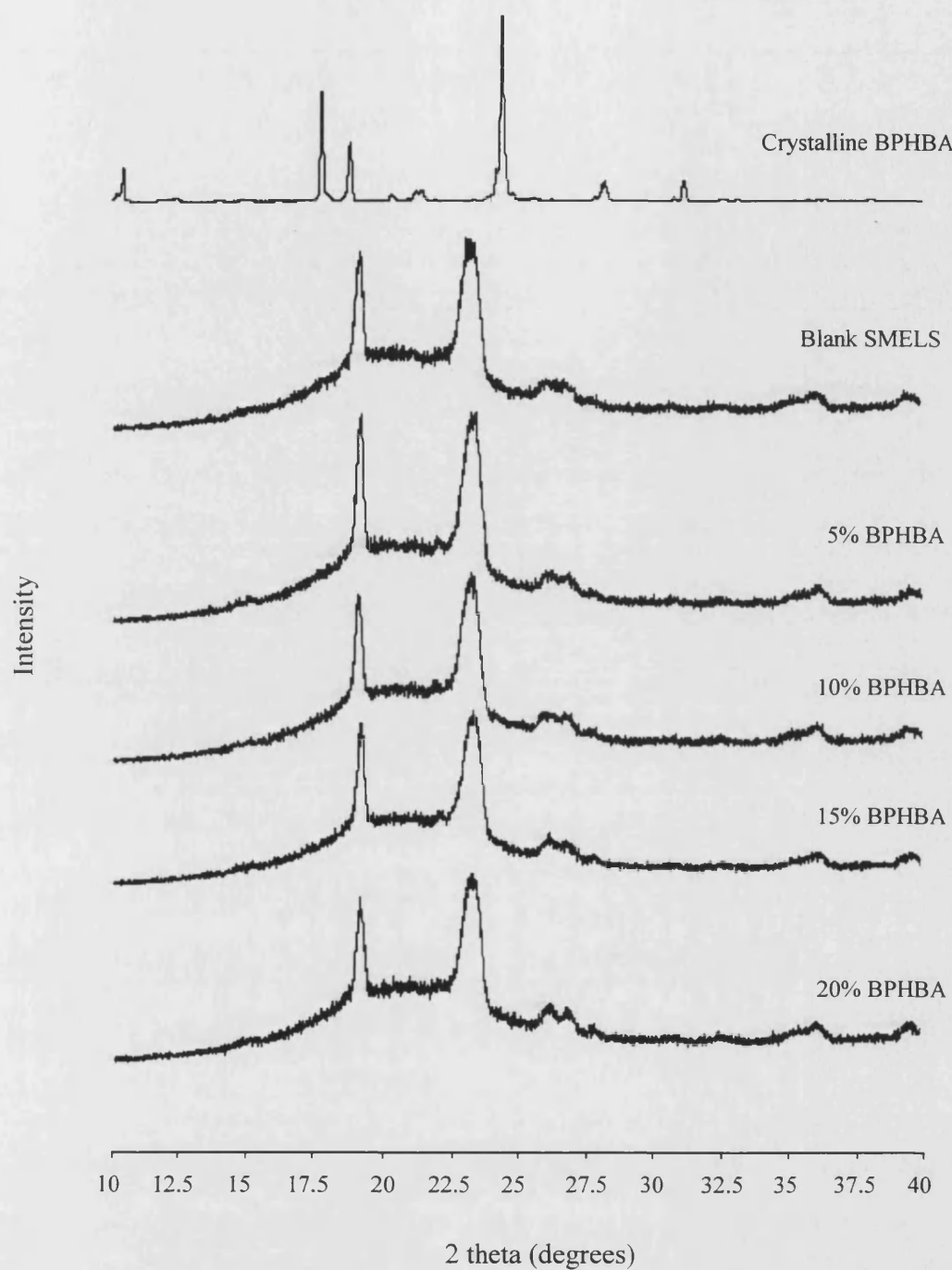
This was evident in our investigation on Butyl-para hydroxy benzoic acid (BPHBA)-PEG 10000 solid dispersion systems. BPABA was used here as a model drug for lipophilic weak base compounds with  $\log p = 3.24$  and  $pK_a = 9.03$ . Figure 4.6 shows the X-ray diffraction patterns for pure BPHBA, PEG 10000 and solid dispersions of PEG with increasing percentages of crystalline BPHBA. The diffractogram of BPHBA showed a number of characteristic peaks at a diffraction angle of  $2\theta$  17.84, 18.87 and 24.5 (the peak occurring at 24.5  $2\theta$  had the highest intensity), suggesting that the drug is present as a crystalline material. The BPHBA crystalline peak occurring at 24.5  $2\theta$  was observed in all BPHBA-PEG solid dispersion systems containing amounts of drug at 10% or more, as indicated by the arrows in figure 4.6. This suggests that at BPHBA concentrations of  $\geq 10\%$  these formulations become contaminated with crystalline material. Absence of the characteristic peaks of crystalline BPHBA in the formulations containing  $\leq 7.5\%$  of drug indicates that the BPHBA was present in these dispersions in the amorphous state.

X-ray diffractograms of pure crystalline BPHBA, blank carriers of *h*-SMELS and *h*-SMELS with increasing amounts of BPHAB are shown in figures 4.7 and 4.8. Formulations of *h*-SMELS are composed of the self-micro-emulsified oil mixture (Miglyol 812/Imwitor 988-Cremophor RH40) at ratios of either 70(10/90)30 or 70(30/70)30 and PEG 10000 at concentrations of 32.5 or 22.5% w/w, respectively. Diffractograms of BPHBA-(*h*-SMELS) dispersions (figures 4.7 & 4.8) were similar to those of the corresponding blank carriers with no evidence of the presence of contaminating crystalline material. Regardless their composition, these new carrier systems (*h*-SMELS) appear to have high loading capacity of the drug in the amorphous state as high as 20% w/w in contrast to  $\leq 7.5\%$  in the case of the



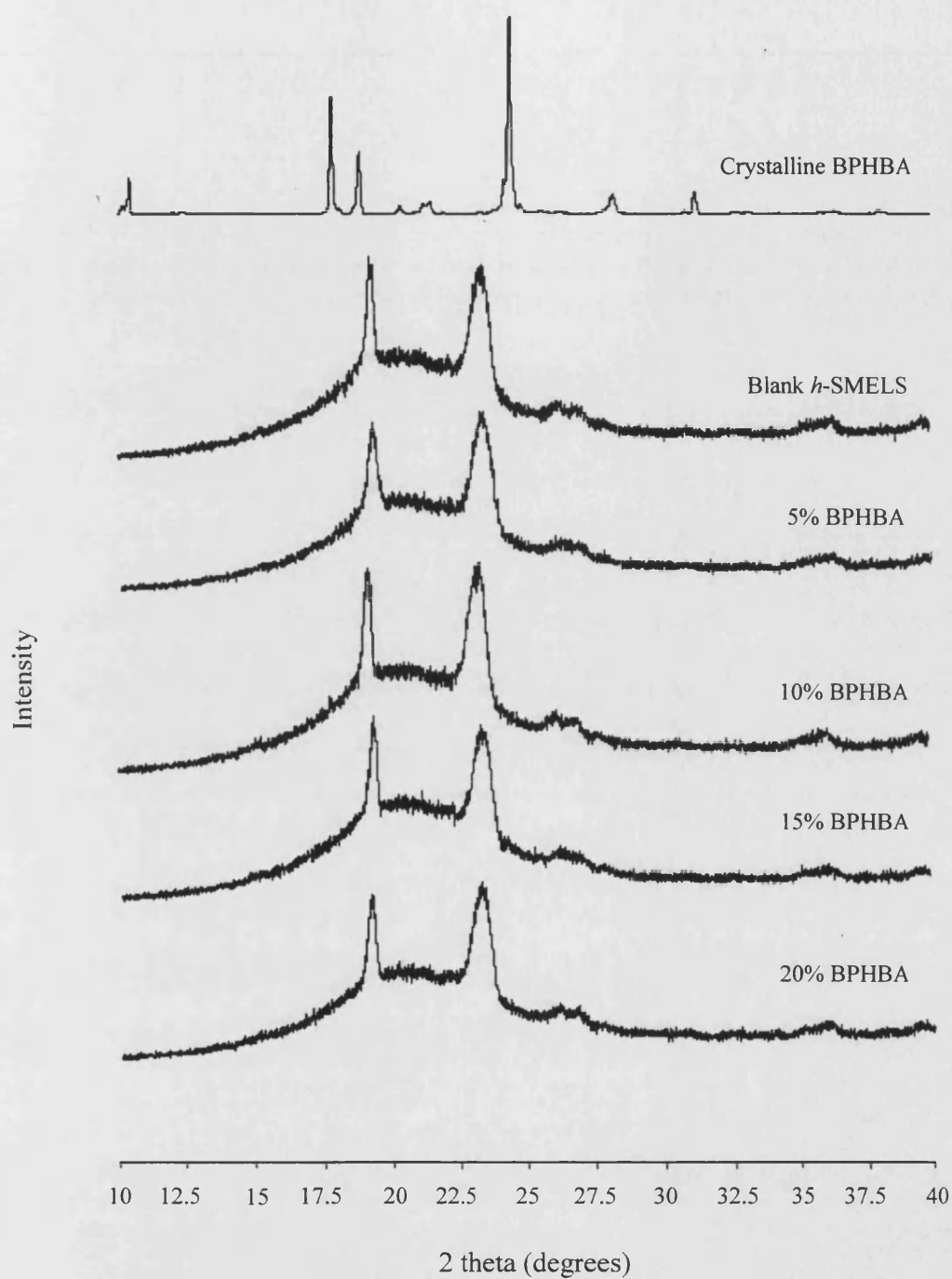
**Figure 4.6:**

X-ray diffractograms of crystalline BPHBA, pure polymeric PEG 10000 and solid dispersions of PEG with increasing amounts of BPHBA (arrows correspond to crystalline BPHBA).



**Figure 4.7:**

X-ray powder diffractograms of pure BPHBA, blank carrier of *h*-SMELS and *h*-SMELS with increasing amounts of BPHBA. Carriers of *h*-SMELS are composed of the oil mixture Miglyol 812/Imwitor 988-Cremophor RH40 at ratios of 70(10/90)30 and the polymeric carrier PEG 10000 at concentration of **32.5%** w/w.



**Figure 4.8:**

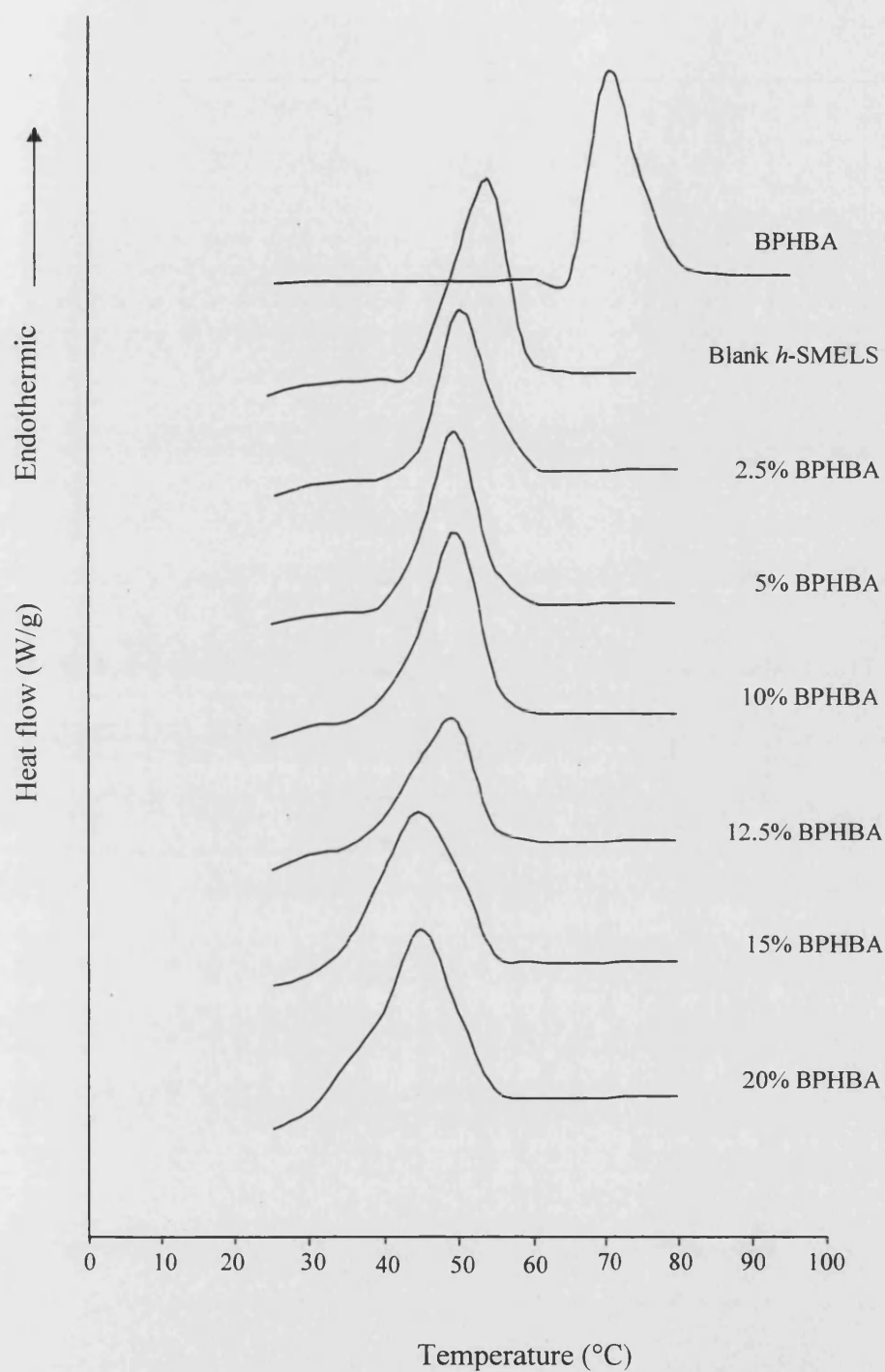
X-ray powder diffractograms of pure BPHBA, blank carrier of *h*-SMELS and *h*-SMELS with increasing amounts of BPHBA. Carriers of *h*-SMELS are composed of the oil mixture (Miglyol 812/Imwitor 988)-Cremophor RH40 at ratios of 70(30/70)30 and the polymeric carrier PEG 10000 at concentration of **22.5%** w/w.

polymeric carrier PEG 10000. This is attributed to the fact that these carrier systems have high oil content in which the compound is highly soluble, the solubility of BPHBA in the oil mixture averages between 16 to 17% w/w.

DSC analysis was also carried out for further physical characterization of these systems in order to confirm results from the XRD. DSC thermograms of pure BPHBA, blank carriers of *h*-SMELS (composed of various oil mixtures with varying concentrations of PEG 10000), and *h*-SMELS with increasing percentages of BPHBA are shown in figures 4.9 and 4.10. Each thermogram of *h*-SMELS based formulations displayed single melting endothermic peak similar to that of the corresponding blank carrier. The endothermic peak attributable to the melting of BPHBA (occurring at  $\approx 65-70^{\circ}\text{C}$ ) was not observed in the spectra of *h*-SMELS based formulation at all amounts of BPHBA used (Figures 4.9 & 4.10). In agreement with the XRD results, the absence of a second endothermic peak at  $\approx 65-70^{\circ}\text{C}$  which corresponds to crystalline BPHBA suggests that the drug was dispersed in the amorphous state in these carrier systems regardless their composition. However, in the case of *h*-SMELS based formulations composed of the oil mixture Miglyol 812/Imwitor 988-Cremophor RH40 at ratios of 70(30/70)30 and 22.5% w/w of PEG (figure 4.10), at high percentages of BPHBA, for example 15% w/w, DSC thermograms produced relatively sharp peaks comparable to the carriers containing oil ratios of 70(10/90)30 and 32.5% PEG at same concentrations of drug (Figure 4.9). As the former carrier system contains relatively higher Miglyol 812 (source of triglycerides), it is anticipated that Miglyol 812 increases the fluidity of these systems and hence enables to solubilise more BPHBA within the formulation. On the other hand, it was observed that the progressive increase of BPHBA in these carrier systems resulted in corresponding decreases in the endothermic energy. This effect is may be due to the increased fluid viscosity when high drug content is introduced into the formulations of *h*-SMELS.

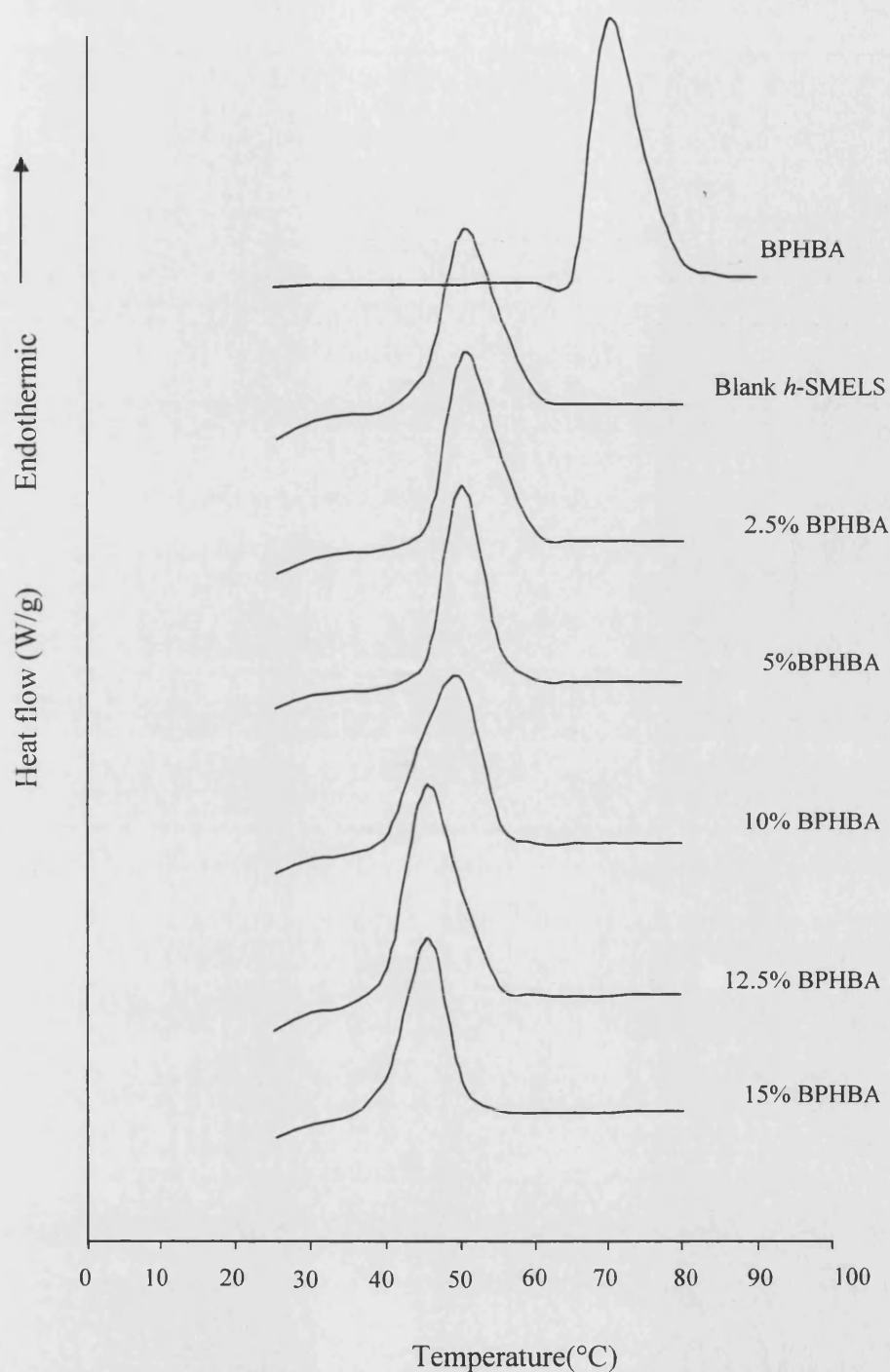
#### 4.4.2.2. *l*-SMELS

These carrier systems are composed of the hydrophobic self-micro-emulsified oil mixture {Miglyol 812/Imwitor 988-Tagat TO} at ratios of 70(70/30)30 and carnauba wax (lipophilic solid carrier) at concentration of 30% w/w. In order to investigate the



**Figure 4.9:**

DSC thermograms of pure crystalline BPHBA, blank carrier of *h*-SMELS and *h*-SMELS with increasing amounts of BPHBA. Formulations of *h*-SMELS are composed of the oil mixture Miglyol 812/Imwitor 988-Cremophor RH40 at ratios of 70(**10/90**)30 and the polymeric carrier PEG 10000 at concentration of **32.5%** w/w.



**Figure 4.10:**

DSC thermograms of pure BPHBA, blank carrier of *h*-SMELS and *h*-SMELS with increasing amounts of BPHBA. Formulations of *h*-SMELS are composed of the oil mixture Miglyol 812/Imwitor 988-Cremophor RH40 at ratios of 70(30/70)30 and the polymeric carrier PEG 10000 at concentration of **22.5%** w/w.

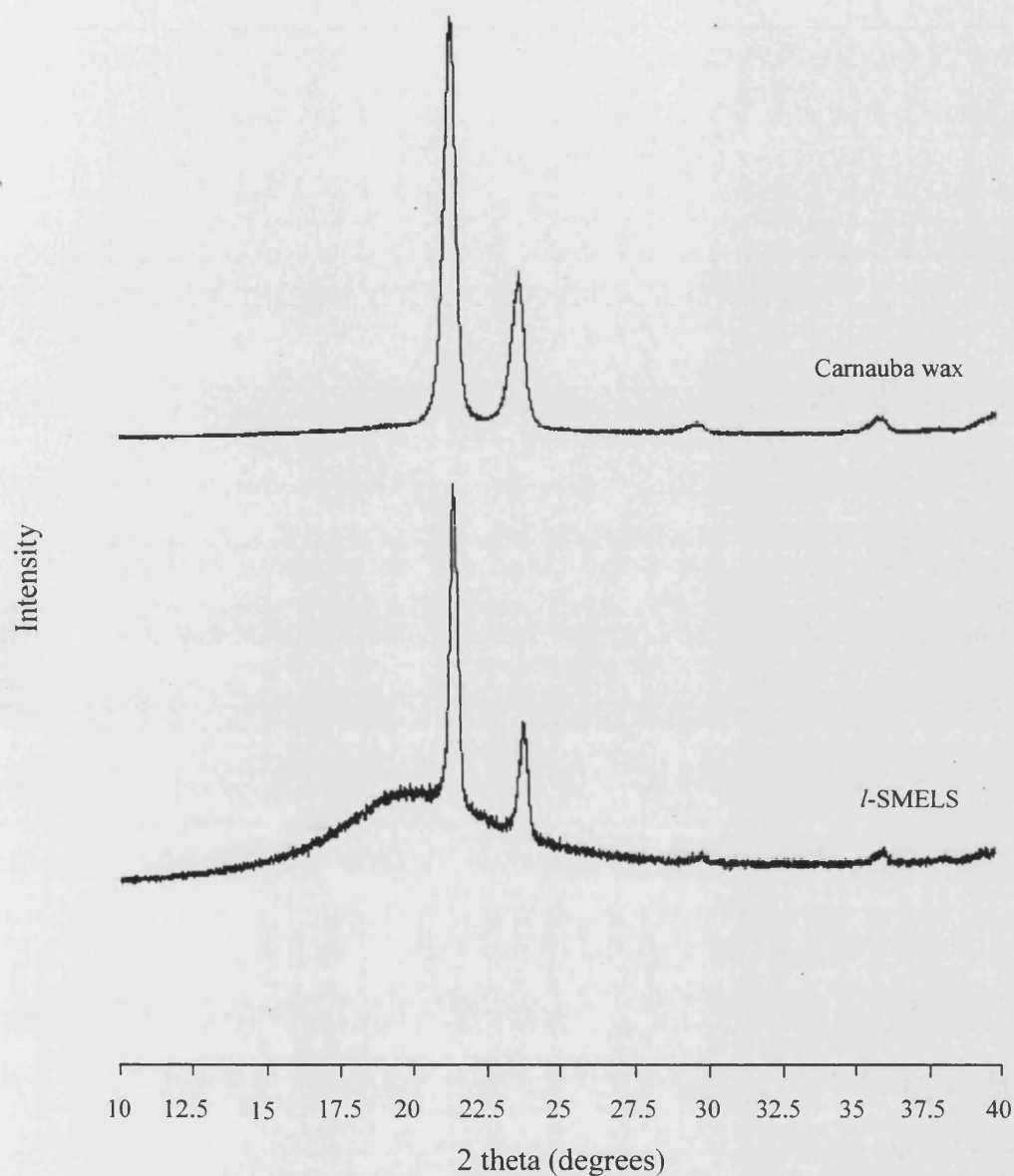
physical nature of these solid vehicles, XRD analysis was carried out. Figure 4.11 shows the X-ray diffraction patterns of pure carnauba wax and the formulation of *l*-SMELS. The X-ray diffractogram of pure carnauba wax reveals two distinctive characteristic peaks at diffraction angles of  $2\theta$  21.43 and 23.75. On the hand, the XRD for the *l*-SMELS based formulation showed patterns similar to that of the pure carnauba wax with same characteristic peaks at 21.43 and 23.75  $2\theta$ . This suggests that these new vehicles are thought to be composed of eutectic mixtures whereby, the self-emulsified oil mixture is contained within the crystalline matrix of the carnauba wax. Furthermore, on dilution with water, the oil mixture in situ tends to move out from the carrier system forming in the aqueous phase fine emulsions, while the carnauba wax is left to disperse as large particles. The presence of carnauba wax may, therefore, influence the release and the dissolution of the self-emulsified oil mixture which contains the active molecule forming what is thought to be a sustained release system. However, bioequivalent study needs to be carried out for further characterization of these carrier systems.

The *l*-SMELS based formulation was mixed with increasing amounts of crystalline BPHBA to investigate the physical state in which the active molecule exists in these carrier systems. The X-ray diffractograms of pure crystalline BPHBA, blank formulation of *l*-SMELS and *l*-SMELS with increasing percentages of BPHBA (5-15% w/w) are shown in figure 4.12. As mentioned earlier the crystalline BPHBA had shown various characteristic peaks of which the highest intensity occurred at 24.5  $2\theta$ . Yet, the X-ray diffractograms of *l*-SMELS based formulations containing increasing amounts BPHBA have shown similar diffraction patterns similar to that of the blank carrier with absence of the characteristic peaks of crystalline BPHBA. This suggests that the drug is present in these carrier systems in the amorphous state without any contaminating crystalline material.

#### 4.4.3. Physical Stability of SMELS

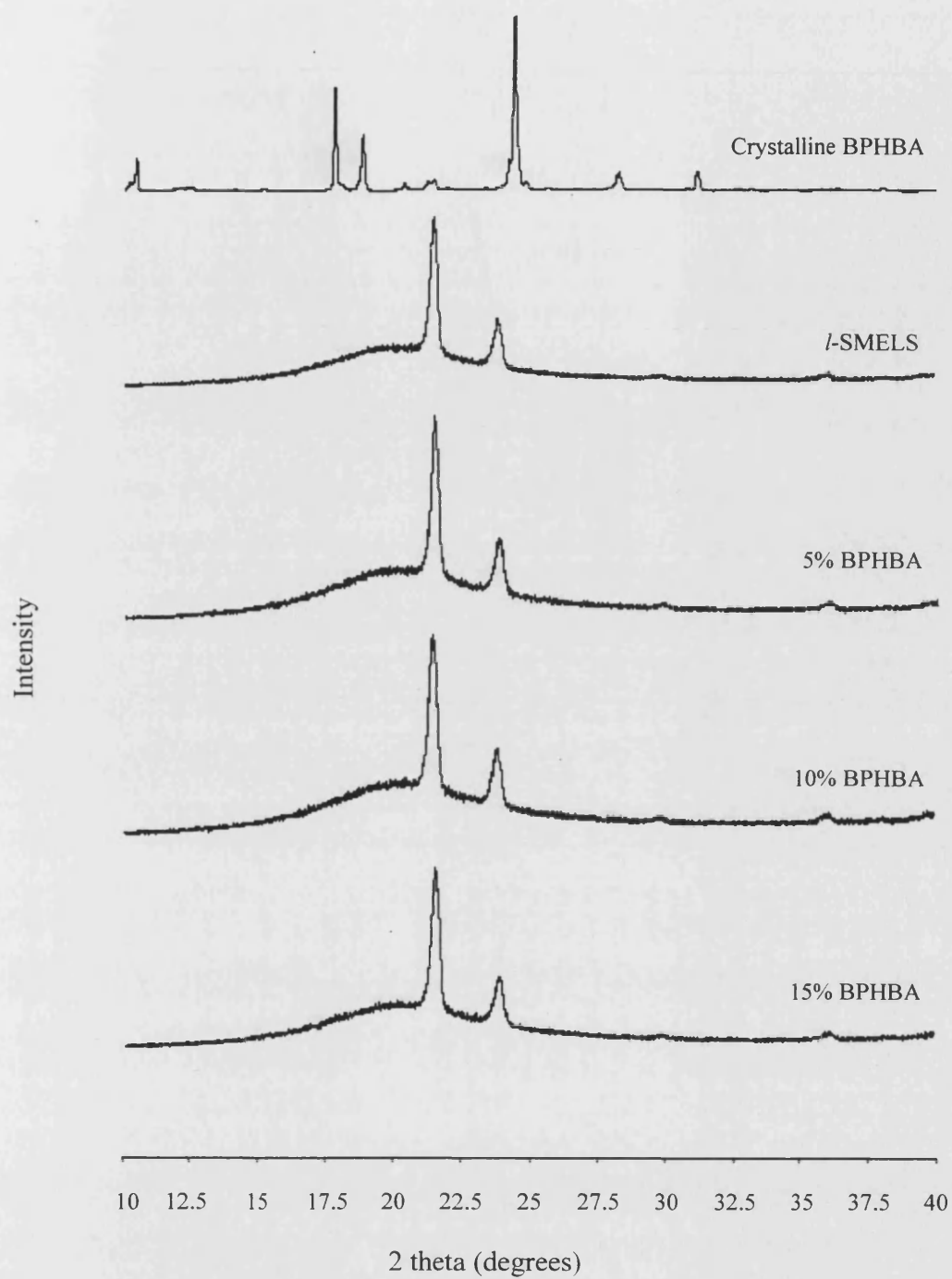
One of the major limitations of the solid dispersion approach is the orientation of the dispersed amorphous drug for crystal nucleation and growth upon aging. Such changes may slow the dissolution rate of the drug in the dispersion and hence result in diminished absorption. As one of the key mechanisms for the enhanced dissolution





**Figure 4.11:**

X-ray powder diffractograms of pure carnauba wax (solid carrier) and a blank formulation of *l*-SMELS. Formulations of *l*-SMELS are composed of eutectic mixtures of the self-micro-emulsified oil mixture of (Miglyol 812/Imwitor 988)-Tagat TO) at ratios of 70(70/30)30 and carnauba wax (solid carrier) at percentage of 30% w/w.



**Figure 4.12:**

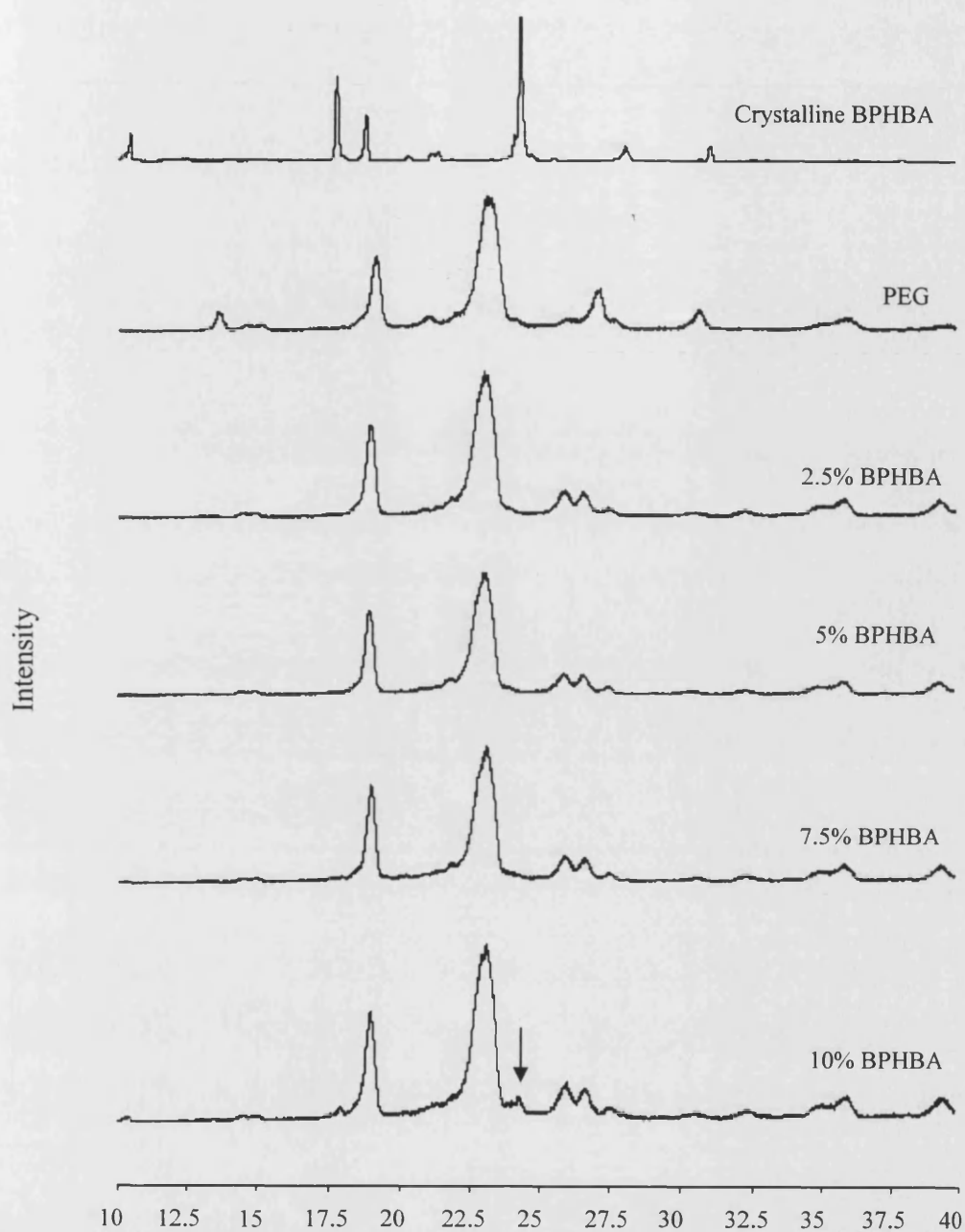
X-ray powder diffractograms of pure BPHBA, blank carrier of *l*-SMELS and *l*-SMELS with increasing amounts of BPHBA.

seen with a solid dispersion, is a change in the physical state of the drug to a higher energy faster dissolving form i.e. metastable amorphous form, then the conversion of drug into a more thermodynamically stable form will ensue in losing the driving force for the increased dissolution rate <sup>[36]</sup>.

The carrier system in which the amorphous drug is dispersed is probably the most crucial element in preventing aging processes. If the carrier is insufficiently viscous or rigid enough to stabilize the drug, or if the carrier is susceptible to aging, the dispersed drug tends to revert to the more stable crystalline form <sup>[34]</sup>. Perng et al. <sup>[36]</sup> reported that following storage at 25°C/60% relative humidity for 1 year, solid dispersions of SB-210661 (lipoxxygenase inhibitor)-PEG 8000 showed a slowing in their dissolution rate, whereas no significant change was observed in the dissolution profile for PVP dispersion. X-ray powder diffraction results indicated that SB-210661 was still amorphous in the PVP dispersion in contrast to the PEG 8000 dispersions whereby, the drug initially existed in a partially crystalline form. In another study by Khoo et al. <sup>[34]</sup> on a solubilizing solid dispersion of Halofantrine (Hf):Gelucire 44/14:Vitamin E TPGS, formulation revealed the presence of crystalline Hf free base after three weeks storage at room temperature.

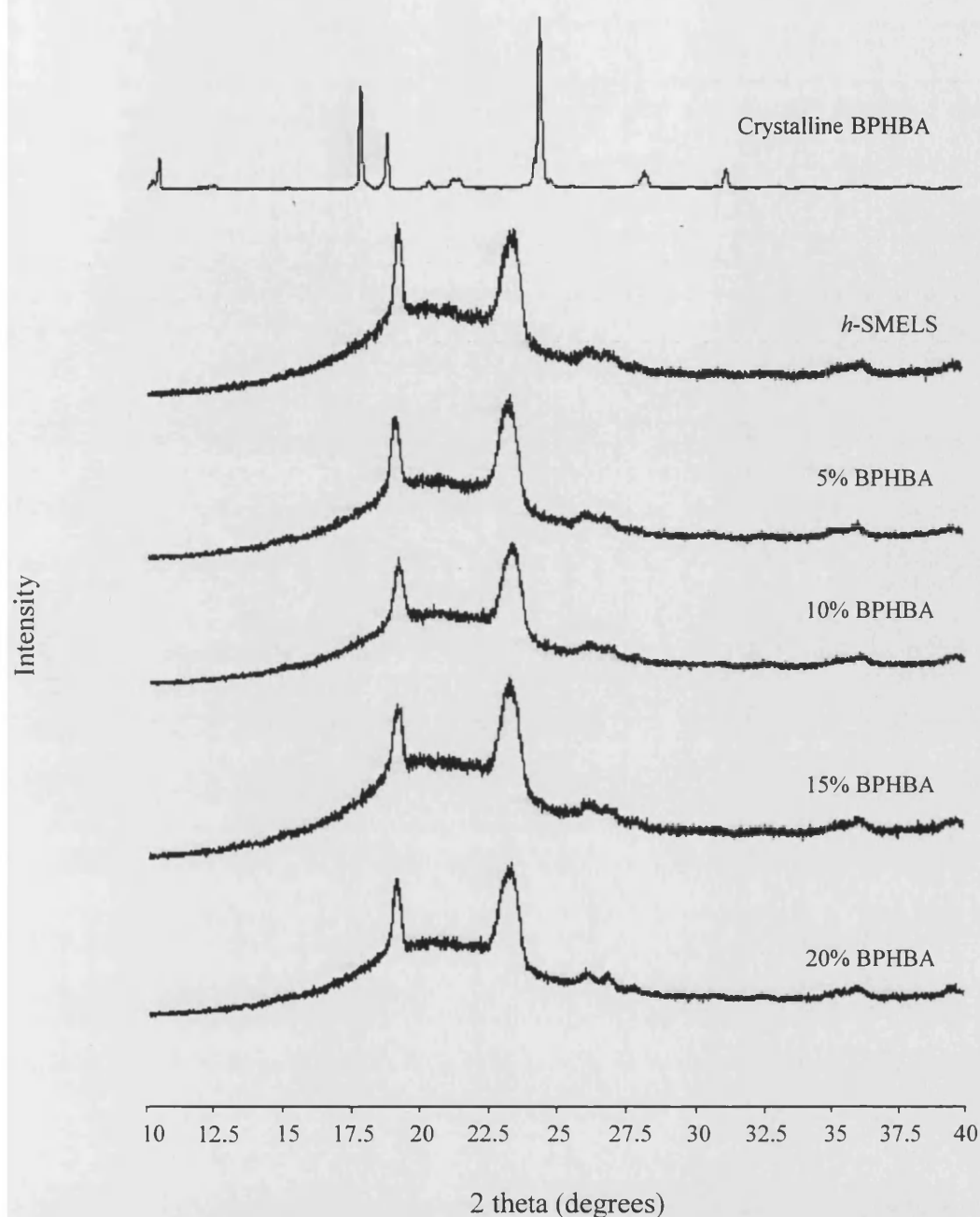
In this study the physical state of BPHBA in dispersions of PEG 10000 or two selected formulations of SMELS was investigated after storage at room temperature (25°C) for two years using X-ray powder diffraction. The X-ray diffractograms of solid dispersions of BPHBA-PEG 10000 upon storing for two years are shown figure 4.13. The presence of crystalline BPHBA could be detected in the formulation when the drug concentration was 10% or more (as the arrow indicates), which is similar to the patterns observed when samples were initially characterized at day zero (figure 4.5). The reason why contaminating crystalline material could not be detected at low amounts of drug (< 10%) upon aging might be attributed to the low sensitivity of the apparatus.

Furthermore, the X-ray powder diffraction patterns of dispersions of BPHBA in vehicles representing *h*-SMELS or *l*-SMELS after storage for two years are shown in figures 4.14 and 4.15, respectively. Those figures showed no evidence of the presence of contaminating crystalline material at all concentrations of drug investigated.



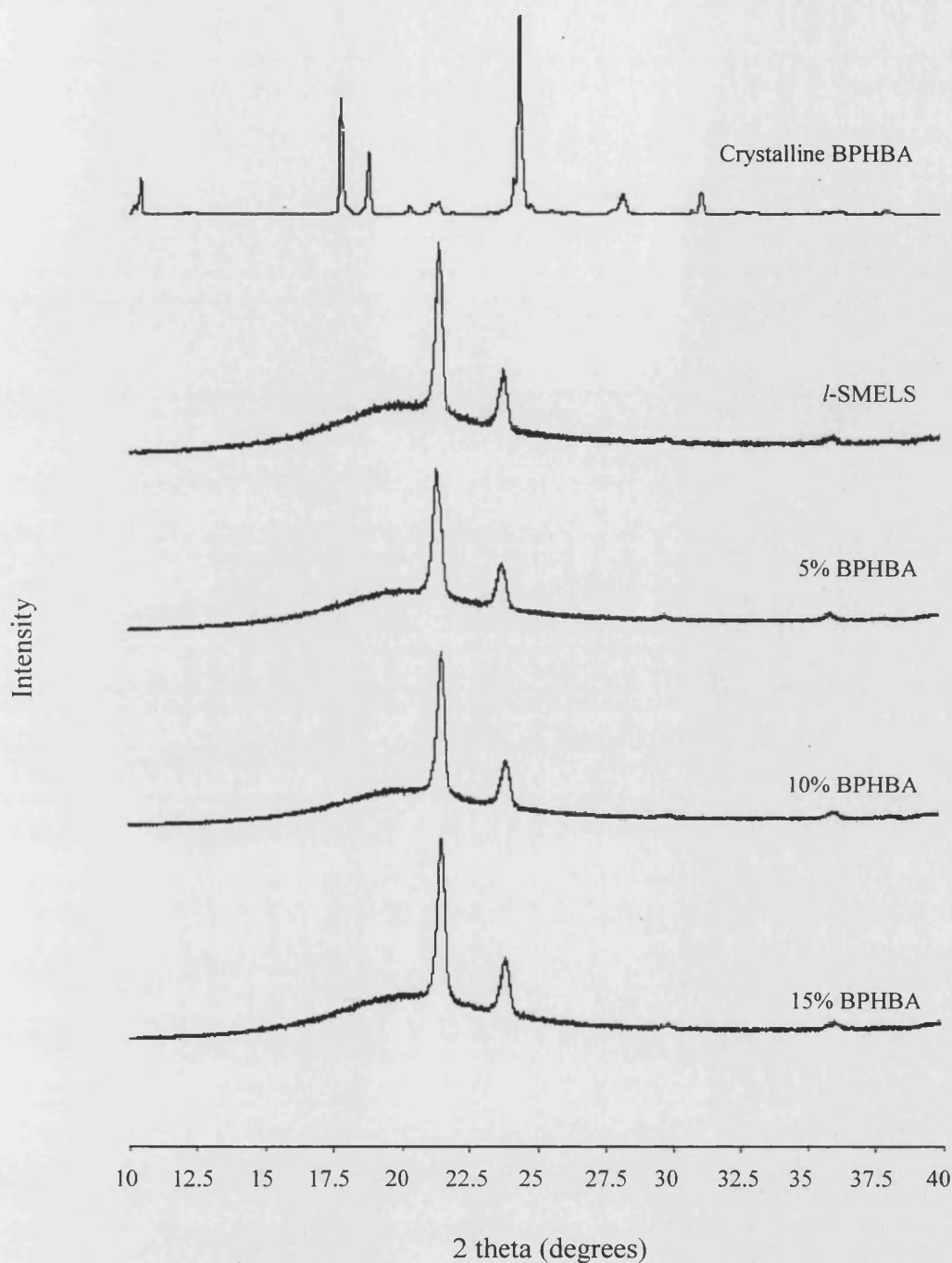
**Figure 4.13:**

X-ray diffractograms of solid dispersions of BPHBA-PEG 10000 after storage for 2 years at room temperature (25°C), arrow corresponds to crystalline BPHBA of peak intensity at 24.5  $2\theta$ .



**Figure 4.14:**

X-ray diffractograms of BPHBA-(*h*-SMELS) dispersions after storage for 2 years at room temperature (25°C), absence of BPHBA crystalline peak at 24.5  $2\theta$  in these dispersions suggests that the drug is still dispersed in the amorphous state. Formulations of *h*-SMELS are composed of the self-micro-emulsified oil mixture of (Miglyol 812/Imwitor 988)-Cremophor RH40 at ratios of 70(30/70)30 and PEG 10000 at percentage of 22.5% w/w.



**Figure 4.15:**

X-ray diffractograms of BPHBA-(*l*-SMELS) dispersions after storage for 2 years at room temperature (25°C), absence of BPHBA crystalline peak at 24.5  $2\theta$  in these dispersions suggests that the drug is still dispersed in the amorphous state. Formulations of *l*-SMELS are composed of the self-micro-emulsified oil mixture of (Miglyol 812/Imwitor 988)-Tagat TO at ratios of 70(70/30)30 and Carnauba wax at percentage of 30% w/w.

The absence of crystalline BPHBA in these systems indicates that the drug is still dispersed in the amorphous state after storing for two years.

These new carrier systems appear to have the potential advantages over the traditional dispersion systems in first; the high loading capacity of drugs to be presented to the GIT in their high energy (metastable) amorphous form and secondly; in their ability to maintain the drug in this highly soluble form and hence prevents the crystal nucleation and growth upon aging.

#### 4.5. Concluding Remarks

Various methods have been used to improve the bioavailability of poorly water-soluble molecules, amongst these approaches are; micronization, solubilization in surfactant systems, complexation by cyclodextrines, solid dispersions and the use self-emulsified lipid technology. Solid dispersion systems have the potential advantage of presenting the drug to the GIT in the metastable amorphous form yet, they have low loading capacity of drugs in the amorphous state and also formulations are susceptible to the aging processes. The most significant improvement in bioavailability of hydrophobic drugs was achieved with self-emulsifying formulations; the development of Neoral™ demonstrates such successful approach.

In this investigation solid dispersion technology with the use of self-emulsifying lipid systems were employed to prepare new solid carrier systems which were identified as solid self-micro-emulsified lipid systems (SMELS) in an attempt to replace the costly and inconvenient soft gelatin capsule forms. Depending on the composition of these carrier systems, they are classified into hydrophilic and lipophilic type vehicles (*h*-SMELS and *l*-SMELS, respectively). Formulations of *h*-SMELS are composed of eutectic mixtures of type III self-micro-emulsified oil formulations (Miglyol 812/Imwitor 988-Cremophor RH40) and varying percentages of PEG 10000, whereas *l*-SMELS are composed of the type II self-emulsified system Miglyol 812/Imwitor 988-Tagtat TO at ratios of 30(70/30)30 and Carnauba wax (solid carrier) at concentration of 30% w/w. The results of this study showed that it is possible with this new type of carrier systems to include relatively high quantities of drug in the amorphous state without the tendency for crystal nucleation and growth upon aging.

## Direction of Future Work

Self-emulsified drug delivery systems (SEDDS) have proved to be one of the most successful approaches in enhancing the bioavailability of hydrophobic molecules. The development of Neoral<sup>TM</sup> exhibits an excellent example of the advances in this lipid technology. Various elements in the composition of the self-emulsified oil mixture are crucial for the design of a successful lipid system. Type of oil (source of triglycerides), the use of mixed mono-/di-glycerides (co-surfactants), type of surfactant and the use of cosolvents are the key factors which can influence the drug solubility in the system, the physicochemical properties of the dispersions after the emulsification of the formulation and the propensity towards the effect of electrolytes present in the emulsification media. The preliminary criteria, however, in deciding on a potential successful oil formulation is the ability of the oil mixture to form fine dispersions on dilution with water. This is done through a tedious work of large screening of various oils and surfactants to obtain self-emulsifiable mixtures. We have found, as other researches, that medium chain triglycerides (MCT) and the use of mixed mono-/di-glycerides (such as, Imwitors and Capmuls) can facilitate the emulsification process. In order to help researchers and formulations scientists in quick selection for reliable oil systems, I intend to screen various oil blends (especially medium chain mixed glycerides) and surfactants in order to correlate physicochemical factors (such as, the polarity of oil blend, surfactant HLB and the chemical structure of the surfactant) with the emulsification process.

In this study we have identified some formulations representing type II lipid class systems which can produce very fine dispersions (50-100nm) with materials which are substantially water insoluble. This type of formulation is valuable as there is no loss of solvent capacity on dilution. This system is composed Miglyol 812 (source of triglycerides), Imwitor 988 (equal amounts of mono and di-glycerides) and Tagat TO (polyoxyethylene-(25)-glyceryl trioleate). Oil blends of Miglyol 812 and Imwitor 988 (i.e. oil-cosurfactant ratio) constitute a crucial factor in the resultant dispersions after the emulsification of the formulation. Miglyol 812/Imwitor 988 at ratios of 70/30 with Tagat TO (surfactant) at concentration of 30% w/w produced optimum dispersions of



particle size <50nm. The mechanistics of emulsification of this system appeared to lack regions of liquid crystalline mesophases but showed high capacity for water solubilization. This suggests that the 'Diffusion and Stranding' is the putative mechanism for emulsification of these systems. Imwitor 988 as polar oil due to high content of monoglyceride acts as a cosurfactant in aiding the emulsification process. Therefore, its inclusion has to be optimized to obtain oil droplets with an overall polarity that matches the HLB value of the surfactant in order to produce good dispersions. Our findings have shown that the inclusion of Imwitor 988 in the Miglyol 812/Tagat TO system has influenced the sensitivity of the oil mixture towards the effects of electrolytes present in the emulsification media. Corresponding to its concentration in the system, Imwitor 988 as polar oil was found to cause depression in the phase inversion temperature (PIT) by shifting the surfactant aqueous solubility towards the oil phase and hence inducing phase separation. The electrolytes present in the emulsification media results in further depression in the PIT which could accelerate phase separation at temperatures far below 37° (emulsification temperature). Furthermore, the inclusion of a cosurfactant with high monoglyceride content such as Imwitor 308 (90% of Glycerol mono- caprylate) instead of Imwitor 988 induced relatively more depression in the PIT as it is considered to be more polar oil. In order to counteract the deteriorating effect of the ionic strength of the emulsification media on these systems, it was suggested to use surfactant systems with relatively high HLB value. In this case, the reduction of the surfactant HLB incurred by the inclusion of polar oil or the presence of electrolytes in the emulsification media will not be substantial to induce phase separation. In collaboration with Goldschmidt we intend to synthesise surfactants which have the basic chemical structure as Tagat TO (polyoxyethylene-(25)-glyceryl trioleate with wide range of HLB values by increasing the number of ethoxy residues. This will produce a surfactant system with a proper and relatively high HLB value which when optimized with Miglyol 812/Imwitor 988 oil blends will produce a formulation that is not sensitive to the ionic strength of the emulsification media.

Last but not least, based on solid dispersion technology and self-emulsified lipid formulations, new solid carrier systems were developed which were identified as solid self-micro-emulsified lipid systems (SMELS). According to the type of solid carrier included and the class of lipid system, those new vehicles were classified into

hydrophilic (*h*) and lipophilic (*l*) SMELS. The polymeric carrier PEG 10000 was included in a hydrophilic type III lipid class system to form *h*-SMELS, whereas carnauba wax was included in a lipophilic type II lipid class system to produce formulations of *l*-SMELS. Results obtained from XRD and DSC have shown that these new carrier systems have potential advantage of high loading capacity of drugs in the high energy amorphous state and are not susceptible to the aging processes. They can maintain the drug in the amorphous form without the orientation for crystal nucleation and growth after storing for a long period. I intend to use a drug which has a pharmacological activity in these vehicles and to carry out dissolution rate analysis study to substantiate results obtained from the XRD and DSC.

Spray congealing is a technique of making microparticles by atomizing a solution or a suspension of drug in a melted carrier; atomization process leads to the formation of melted droplets which then solidify upon contact with air <sup>[37]</sup>. I would like to use the spray congealing technique in preparing microparticles of dispersions of SMELS and active compounds which might have the potential to be the bases for table formulations.

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